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Centrifugal Microfluidic Platform for Solid-Phase-Extraction (SPE) and Fluorescence Detection Applications

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CENTRIFUGAL MICROFLUIDIC PLATFORM FOR SOLID-PHASE-EXTRACTION (SPE) AND FLUORESCENCE DETECTION APPLICATIONS

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The Department of Mechanical and Industrial Engineering

by

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Abstract

Solid phase extraction (SPE) is a widely used method to separate and concentrate the target molecules in liquid mixture. Traditional SPE has to be conducted in the laboratory with professional equipment and skilled operators. The microfluidic and 3D printing technology have opened up the opportunity in developing miniaturized automatic instruments. The main contribution of this research is to integrate the SPE process on a novel centrifugal platform. Various valves are applied on the platform to help control the aqueous sample and reagents in the cartridge.

First, a centrifugal microfluidic platform was built for automatically detecting trace oil pollution in water. Mechanical valves were used to control the flow of the reagents and water sample in the device. The prototype of the proposed system was fabricated using 3D printing technology and tested with 10 ppm standard oil-water mixing sample. Different stationary sorbents were tested and compared and the 3D printed porous polymer showed the best performance. The experimental results had proved that the detection sensitivity of the water-oil mixture sample can be significantly enhanced after it was enriched using the centrifugal microfluidic platform.

Then, a truly three-dimensional (3D) microfluidic cartridge for SPE was fabricated and tested on the centrifugal platform. A novel gravity valve was designed and applied in the centrifugal cartridge. Unlike the traditional capillary valves, which can only be sequentially opened via increasing the spinning frequency, the gravity valve was more flexible. Gravity valves, traditional capillary valves, and Coriolis switching valves were integrated in one cartridge to manipulate reagents and the sample involved in the SPE process. All the valves can be simply controlled by the rotating direction and speed of the cartridge. The prototype microfluidic cartridge was 3D printed and experimentally tested. Anthracene, which was one of the Polycyclic Aromatic Hydrocarbons (PAHs), was used as the target analyte and tested with the presented design. C18 was used as the stationary sorbent and fluorescence method was adopted to detect anthracene. The original testing samples
of anthracene were 1 µg/L (1ppb), 5 µg/L, and 10 µg/L (anthracene/water), respectively. The volumes of testing sample and eluent (nonane) were 10 mL and 1 mL respectively. Experiments showed that the prototype device performed well and fluorescence intensities after SPE were 7 to 11 times of the original sample. Perylene of 1 µg/L (perylene/water) was also tested on the platform and the integrated fluorescence intensity after SPE was 8.3 times of the original sample.

Finally, solid phase extraction and fluorescence detection experiments were conducted for the crude oil-containing seawater samples using the prototype device. Oil-seawater samples with oil concentrations of 10 ppm, 5 ppm, and 2 ppm were prepared respectively. The volumes of oil-seawater mixed sample and eluent (nonane) were 10 mL and 1 mL respectively. The integrated fluorescence intensities of the eluent after SPE were measured to be about 4.8 to 6.9 times of the oil-water mixed samples. More importantly, the SPE process eliminated the interference of the CDOM in the seawater.
Chapter 1. Introduction

The BP oil spill accident occurred in 2010 raised people’s concern of water pollution. The conventional methods to monitor the environment water require the researchers to do the sampling first, and then transport the sample to the laboratory to conduct analysis. It is a time-consuming and laborious process, in addition to the risk of contamination during the transport and storage. Very few portable instruments are available for on-site monitoring, and they are usually huge, complicated to operate, and expensive.

In the past decade, the concept of “lab-on-a-chip” has become very popular in biological, analytical, and biomedical fields. As microfluidic technology developing dramatically, microfluidic system shows many advantages. For example, the time and reagent consumptions during the analysis process can be significantly reduced due to the scale effect. As a result, cost of each detection is also reduced. What’s more, the microfluidic method often provide much better sensitivity. In addition, the microfluidic device can significantly reduce the size and weight of the equipment or system, and make it possible for on-site monitoring. Therefore, microfluidic method is a very promising approach for the environmental pollutant detection.

Centrifugal microfluidic platform, which is also known as “Lab-on-a-CD”, or LOC, is an important branch of “lab-on-a-chip” technologies. It includes a CD-like microfluidic platform that with all functional components (such as channels and chambers) integrated on it, and a motor that is used to drive the “CD” to rotate in sequence of precisely controlled frequencies. The centrifugal microfluidics can accomplish many operations, such as separating, mixing, metering, reacting, and detecting, etc. Many efforts have been made to develop “Lab-on-a-CD” systems in biomedical field, such as blood analysis and immunoassay. In recent years, researchers have begun to apply the centrifugal platform in the fields of environmental monitoring and other applications.
1.1. A brief introduction of centrifugal microfluidics

1.1.1. Principle and merits

The first centrifugal fluidic platform was developed in 1969 [1]. Since then, numerous studies have been conducted to adopt the technology in biological and medical analyses. Most of these centrifugal instruments are conventional sized ones. As the microfabrication technology have been developing in recent decades, it has become feasible to integrate many components onto a small device, including fabricating large number of microfluidic components onto a single microfluidic chip. These technical progresses have eventually led to the development of the so called “lab-on-a-CD” technology, a main branch of lab-on-a-chip.

The basic idea of the centrifugal microfluidic platform is to transport liquid samples from the inner reservoirs to the outer ones through microchannels connecting them under the actuation of centrifugal force generated by rotational movement of the CD. Coriolis force is another important force exists while the disc is rotating and generates secondary flow phenomena [2, 3]. Actually, it is worth to mention that both the centrifugal force and the Coriolis force are pseudo-forces which are helpful in solving practical problems. When the disk rotates around an axis perpendicularly passing through the center of the disk, the centrifugal force always acts radially outward, and the Coriolis force always acts perpendicular to both angular velocity $\omega$ and the centrifugal force. Figure 1.1 shows a schematic diagram of forces acting on a spinning disc [2]. And the equation (1.1) and (1.2) shown below gives the numerical computation of centrifugal force and Coriolis force respectively. In the formula, $m$ is the mass of the droplet, $r$ is the distance of the droplet to the disc center, $v'$ is vector of the linear velocity of the droplet relative to the rotating platform, $\omega$ is vector of the angular velocity of the rotating platform.

$$F_c = -m \omega \times (\omega \times r),$$  \hspace{1cm} (1.1)
Figure 1.1. Schematic diagram of forces acting on a spinning disc [2].

\[ F_{co} = -2m \omega \times v'. \] (1.2)

The centrifugal microfluidic platform has many unique advantages [2]. Firstly, it is a pump-free system and it can achieve pulse-free transport of liquids. Secondly, the processed volume on the platform can vary from milliliters to nanoliters with almost no residual. Thirdly, by proper designing the channels and other fluidic components on the platform and control the rotation speed, micro-valves and sequential control of multiple fluid samples can be achieved. In addition, the platform can be easily operated and the compact disk used on the platform can be rapidly and inexpensively produced with biocompatible materials such as PDMS and PMMA [4, 5, 6]. Because of these attractive advantages, many researchers are working on applying the technique in fields of nucleic acid analysis, immunoassays, clinical chemistry, cell handling, water analyzing, etc. [2]. Some of the research achievements are already commercialized [7].

1.1.2. Technology review

In microfluidic systems, pumps are commonly used to drive the samples and reagents to flow in the fluidic chips while valves are frequently used to control the flow directions
and release sequence of the liquids in a fluidic system. Therefore, pumps and valves are two of the most important components in most microfluidic systems.

- **Inward pumps**

In a centrifugal microfluidic platform, a motor is used to drive the fluidic disc to rotate. The rotational motion of the disc generates a centrifugal force which helps to actuate the liquid in the disc to move outward. On one hand, this method has the advantage to provide driving forces for multiple parallel microfluidic testing systems at the same time with low residuals. On the other hand, the centrifugal force has the disadvantage that the liquid flow can only be driven one way only to move outward to the edge of the disc. However, complex testing tasks often require the liquid samples to be transported in both directions, both radially outward and inward. To achieve this purpose, many inward pumping techniques have been developed for the centrifugal fluidic applications. Most of these technologies reported in the field are based on pneumatic pumping methods, which driving and replacing the aqueous reagents with gases (usually air). For instance, with ventless chambers and external heating source, inward pumps based on thermal expansion can be realized [8, 9]. Figure 1.2(A) shows the working principle of two thermal pumps (push pumping based on air expansion during heating and pull pumping based on air compression during cooling) [9]. Pumping by electrolytic gas generation as shown in Figure 1.2(B) is another possible solution [10]. However, external electrical connections are required for electrolytic gas generation. Another inward pump is also developed based on air compression [11]. At high spin frequency, the air in a ventless chamber can be compressed by the liquid. At low spin frequency, the air expands and drives the liquid flowing inward. Other than the pneumatic methods presented above, inward pumping can be accomplished with squeezing. As shown in Figure 1.2(C), if the microfluidic structure is made with elastic material such as PDMS, inward pumping can also be realized via squeezing method with a special designed mechanical system mounted on the centrifugal system [12].
• **Valves**

Based on controlling and actuation methods, valves on the centrifugal microfluidic platform are grouped into passive valves and active valves. Passive valves are solely actuated by centrifugal force, which is determined by their locations as well as the spinning frequency of the platform. Capillary valves, hydrophobic barrier valves, and burstable seal valves are some typical passive valves widely used in centrifugal fluidic systems [2]. Valves can also be classified as normally-closed and normally-opened ones. Most of the passive valves used in centrifugal fluidic platforms are the normally closed ones. They are turned to open by simply increasing the spinning frequency to their corresponding burst frequencies (the spinning frequency of the disc at which the valve switch its status from close to open). In contrast, siphon valves are normally-opened during an intermediate static status or low spin frequency status [13]. The siphon valves will be closed while the spinning speed of disc is high.

On the other hand, active valves are controlled and actuated by external means other than centrifugal force. Melting [14, 15] or freezing [16] barrier material in the fluidic channel
is the most commonly used method. These heating/cooling source includes stationary halogen lamp [14], mobile laser diode [17], thermo-electric module [16], laser [18], and hot air gun [15] etc. The active valves can be either normally closed or normally open.

- **Other functions and operations**

  Other than pumping and valving, functions and operations can be realized on the centrifugal microfluidic platform include but not limited to the following: sample and reagent loading, metering and aliquoting, mixing, separation, droplet handling, and detection [2], etc. There are different approaches to accomplish these functions and operations. Traditional methods are mainly based on the inherent property of the centrifugal platform. For example, taking advantage of centrifugal force, serum and blood cells can be separated since they have different density [19]. To achieve more complex manipulation, external powers (such as heating source, laser, electrodes, etc.) are applied on the centrifugal platform. For example, cartridge integrated with electrodes can separate specific cells via di-electrophoresis method [20, 21].

1.1.3. **Application in environment water monitoring**

Currently, most complex environment water analyses are manually sampled and then transported to labs for further analyzing. The entire process is labor- and time-consuming. Centrifugal microfluidic platform maybe a potential solution for on-site monitoring. Various centrifugal microfluidic cartridges were developed to detect the ions, pathogens, and organic contaminants in water, or analyze the PH and turbidity of the water [2]. For example, Czugala et al developed a cartridge for centrifugal platform to measure turbidity and PH of the river water [22]. Hwang et al reported a Lab-on-CD cartridge to detect the nutrients (ammonium, nitrite, nitrate, silicate, and orthophosphate) in water. A few water analyzing systems based on Lab-on-CD are even commercialized. For example, LaMotte Water Link Spin Touch test kit is a photometer based ion and PH analyzer. To detect the trace organic pollutants in water, pre-concentration of the test samples may be necessary. Some researchers tried to integrate the analyte enrichment process on to the centrifugal platform.
Lafleur et al integrated a minimized solid phase extraction (SPE) column in the CD like cartridge to adsorb the analyte in water [22, 23]. Kazarine et al demonstrated a Lab-on-CD system for multi-cycle liquid-liquid extraction [24]. An external pneumatic system was applied to help realize the recirculation on centrifugal platform.

1.2. Fluorescence detection

1.2.1. Theory of fluorescence

Fluorescence is a form of luminescence. When a substance is irradiated with incident light of certain wavelengths (usually ultraviolet or X-ray), it may absorb the incident light and emit light in wavelengths different from those of the incident one (usually in the visible light range). Figure 1.3 is a typical Jablonski diagram, which shows the status of the fluorophore molecule and explains the principle of fluorescence. In the diagram, S0 represents the ground state, which has the lowest electronic energy level. S1 and S2 represent the first and second excited states, which have difference electronic energy levels. A series of vibrational energy levels exist in each electronic energy level, and they are marked as 0, 1, 2, etc. At room temperature, the fluorophore molecules are usually in ground state S0. However, they can absorb irradiations with certain wavelengths, and jump to excited states with higher energy levels in about $10^{-15}$s. Then, the molecules relax to the lowest vibrational level (level 0) of the first excited level S1 very quickly (about $10^{-12}$s). This process is internal conversion and no light emits. Excessive energies are lost as heat. Then, the molecules go back to the ground state S0 in about $10^{-9}$s, but with different vibrational levels. Light emitting occurred during this process with the fluorescence spectrum determined by the probabilities that the molecules return to the different vibrational energy levels. Finally, all the molecules relax to the lowest vibrational energy level of S0 and reach the thermal equilibrium again.

If the molecules in S1 state experience a spin conversion, they will go to the first triplet state T1. The spin conversion from S1 to T1 is called intersystem crossing. Light emission will also occur when the molecules go back to S0 from T1, but the wavelength will be
shorter than fluorescence. This phenomenon is termed phosphorescence. The duration of phosphorescence is usually between $10^{-6}$ s and $10^{-3}$ s.

![Jablonski Diagram](image)

Figure 1.3. Jablonski diagram shows the principle of fluorescence and phosphorescence.

1.2.2. Application in environment water monitoring

Though most compounds can absorb UV or visible light, only a few of them can emit fluorescence. And most of the fluorescent compounds have aromatic rings in their molecule structure. In addition, each fluorophore has its own excitation and emission spectrum. As a result, fluorescence detection can be applied to detect certain fluorescent pollutant in water. For example, polycyclic aromatic hydrocarbons (PAHs) are organic compounds consisting of two or more aromatic rings. Figure 1.4 shows the molecule structures of several typical PAHs. PAHs are widely existing in the environment and harmful to human’s health. Their solution can emit fluorescence when excited by UV light. Basically, more aromatic rings in PAHs lead to longer emission wavelength. Thus, fluorescence detection can be used to monitor PAHs in water. Figure 1.5 shows a simple setup for fluorescence detection. As shown is the diagram, the testing sample is loaded in a cuvette with four clear sides. UV light is introduced from one side of the cuvette with an optical fiber. PAHs in the sample solution will be excited by UV light and emit fluorescence. The fluorescence is collected by another optical fiber perpendicular with the incident UV light and introduced
to a spectrometer connected to a computer. As a result, the fluorescence spectrum will be displayed on the computer for further analysis. When the concentration of the fluorophore is low, the fluorescence intensity can be calculated with the following formula:

\[ I_f = 2.3\Phi_f I_o \epsilon bc, \]  

(1.3)

where \( \Phi_f \) is a proportionality constant determined by the property of fluorophore and measuring instrument, \( I_o \) is the incident photon flux (incident light intensity), \( \epsilon \) is molar absorptivity, \( b \) is the path length of the incident light, \( c \) is the concentration of the testing sample. It can be found from the formula that fluorescence intensity is proportional with the concentration of the sample if all other conditions remain the same.

It is worth to mention that PAHs are important components of crude oil, too. As a result, the crude oil can also absorb UV light and emit fluorescence. Actually, the Turner-10AU field portable Fluorometer (T-10AU), a precise instrument based on fluorescence detection, was widely used in monitoring the coastal water system after the oil spill disaster occurred in Gulf of Mexico.

![Molecule structures of several typical PAHs.](image)

Figure 1.4. Molecule structures of several typical PAHs.

1.3. **Solid Phase Extraction (SPE)**

In a certain range, the fluorophore concentration and fluorescence intensity are roughly proportional. Therefore, sample enrichment is preferred to enhance the detective sensitivity
before fluorescent detection. For example, if the fluorophore is extracted to enrich its concentration to three times of the original one, the sensitivity of the overall measurement can be improved by about three times with the same sensing device and signal processing circuit.

Many efforts have been made to find effective method to extract the analyte from the sample. Liquid-liquid extraction (LLE) is one of the most common technologies. Taking advantage of the solubility difference, fluorophore molecules can be transferred from water to certain organic solvents. Traditional LLE process is simple but usually tedious with low selectivity. Supercritical fluid extraction (SFE) [25] is an advanced sample enriching method with high selectivity and speed. However, high pressure is involved in the process and complex apparatus is required. Solid phase extraction (SPE) is one of the most widely used approach for analyte enrichment. With appropriate stationary sorbent and eluent, analyte will be finally concentrated in the eluent. Particularly, Stir-Bar Sorptive Extraction (SBSE) is a very popular method. The basic ideal of SBSE is as following: immerse a magnetic bar covering with polymer (usually PDMS) film in the testing sample and rotate the bar with external magnetic field to make the polymer film adsorb analyte molecules in the sample solution, then put it into organic solvent for desorption. This method has good sensitivity but the process is difficult to be automatized [26]. Solid-phase micro-extraction (SPME) is quite similar with SPE. But microstructures like PDMS coated fiber is used as the sorbent. It’s a low cost and rapid method but the sensitivity and effectiveness need to be improved [26, 27].
Chemical polarity is an important property of molecules. Substances sharing the same chemical polarity (polar, or nonpolar) usually demonstrate high affinity or solubility to each other. Solid Phase Extraction (SPE) is a very common technology used to separates analyte from the sample solution based on the polarity difference. Figure 1.6 shows the working principle of the reversed phase Solid Phase Extraction (SPE). First, the conditioning buffer goes through the stationary sorbent (usually in powders) to activate to the sorbent. Then, the sample solution with analyte molecules is loaded and driven to flow through the sorbent. During this process, solvent of the sample solution (usually water) directly passes through the sorbent and goes out from the outlet while analyte molecules are adsorbed on the surface of the sorbent particles because they have the similar polarity. Finally, the eluent is loaded and flows through the sorbent to extract the analytes. The volume ratio of the sample solution and the eluent would be the theoretical enrichment coefficient of the SPE process.

![Figure 1.6. Working principle of reversed phase Solid Phase Extraction (SPE).](image)

In addition, a washing process is often added between sample loading and elution. During this process, a selected solvent will pass through the sorbent and bring out the impurities and interfering substances but leave the analyte molecules in the sorbent.

Though traditional SPE method has been widely used for decades, it usually has to be conducted in the lab with various professional instruments and skilled operators. As a
result, it’s not suitable for on-site detection. And a portable automated SPE instrument is highly demanded.

1.4. 3D printing technology

In the last decades, due to the significant progresses in microfabrication technologies such as UV lithography and PDMS molding technique, the microfluidic technologies have found wide applications in various fields. In recent years, 3D printing technology has experienced a phenomenal development and become very attractive for many applications. The 3D printing technology has the advantages of the fast turn–around time in prototype and production, unique capability of producing complex structures, and the flexibility in selections of different materials for specific applications. As a result, the technology has found many applications in microfluidics field. A wide varies of microfluidic devices with different requirements have been 3D printed [28]. For example, Stereolithography (SLA) is often favored for its high printing resolution and compatibility with transparent materials. Thus, it can be used to make microfluidic chips with active parts (such as valves) [29, 30], microfluidic interface (MFI) [31], and pathogen detecting chips [32], etc. However, its application in complex microfluidic system is limited because it is very difficult to remove the residual resin in slender micro-channels. On the other hand, Fused Deposition Modeling (FDM) is a low-cost 3D printing technology that can be used to construct complex 3 dimensional structures with flexible supporting materials. Figure 1.7 shows the working principle of the FDM 3D printing technology. In recent years, the researcher tried to apply the FDM 3D printing technology in fabrication of centrifugal microfluidic systems [33, 34, 35, 36]. Significant progress has been made.

1.5. Scope of the research

The research work in this dissertation focuses on the development of a portable 3D printed centrifugal microfluidic platform for SPE and fluorescence detection. Its heart is a disposable microfluidic cartridge for analyte pre-concentration. The cartridge will be inexpensively 3D printed and mounted on a shaft driven by a programmed servo motor. The
centrifugal force is adopted as the driving force for all liquids in the cartridge. All the valves are simply controlled by the spinning frequency and direction of the platform. This approach helps to eliminate the requirement of external pumping and accessories. After the SPE process, the concentrated analyte will be collected in the detecting chamber of the cartridge. Fluorescence detection can be directly conducted without taking the sample out to a cuvette.

In Chapter 2, a centrifugal microfluidic platform was designed and fabricated for enriching and detecting trace crude oil in water. A mechanical valving system mounted beneath the fluidic cartridge was used to control the liquids in the cartridge.

In Chapter 3, instead of using mechanical valving technology, a truly 3-dimensional microfluidic cartridge based on a novel gravity valve and multiple capillary valves were used in controlling the sample fluids and reagents. The gravity valve could provide a more flexible spin frequency for reagent releasing, which helped enhance the performance of SPE.
In Chapter 4, the 3D cartridge presented in Chapter 3 was applied to enrich the crude oil in oil-seawater mixed samples. The SPE process was used not only to enrich the oil in sample, but also helped to eliminate the interference of the CDOM in the seawater.

These centrifugal microfluidic systems are easy to operate and maintain. They can be potentially applied to on-site environment water monitoring.
Chapter 2. A 3D Printed Centrifugal Microfluidic Platform for Spilled Oil Enrichment and Detection Based on SPE

2.1. Introduction

In 2010, the BP offshore crude oil spill accident in the Gulf of Mexico shocked the world. It was identified as the largest marine oil spill accident so far and 4.9 million barrels of oil was estimated to be discharged. To breakdown the spilled oil, about 1.4 million US gallons chemical dispersant was used. Although great efforts had been made to control and eliminate the pollution caused by the spilled oil, the massive residual oil and dispersant still have a long-term effect on the coastal water [37]. Research shows that the spilled oil was toxic to a wide range of organisms and its effect might last for years [38]. For example, dolphins in Barrataria Bay suffered lung disease caused by the oil since 2010 [39]. On the other hand, though the usage of dispersant helped degrade the spilled oil, it also enhanced the bioavailability and ecotoxicity of the oil to certain habitants [38].

Some studies shows that the degrading of the spilled oil in seawater is not linear and may not follow researcher’s expectation [40]. The concentration of the oil varies largely by time and location. As a result, on-site monitoring devices are strongly needed for rapid collection of real-time data. What’s more, some oil slicks were not breakdown by the dispersant. They might have entered the coastal water and inland water system due to tides and waves [41]. The spilled oil tends to dissolve and degrade slower in fresh water than in seawater. Therefore, emulsion of ‘oil in water’ might be formed in vast areas of marshes and wetlands along the north coast of Gulf of Mexico. So portable and efficient technologies are therefore highly desired to monitor the residual oil pollution in water [42].

The fluorescence detection method is one of the most popular method adopted to determine existence and concentration of the oil in water. Polycyclic aromatic hydrocarbons (PAHs) are important components of crude oil. When an excitation light is directed to
oil sample, PAHs molecules absorb the excitation light and jump from the ground state to the excited state. When the excited molecules return to the ground state, part of the absorbed energy is lost by emitting fluorescence. In practice, crude oil samples are usually stimulated with UV source, and emit light in the range from 400 nm to 650 nm [43, 44].

To monitor the concentration of oil content in water, enrichment of the samples is often the first step in order to enhance the detecting sensitivity. However, the traditional analyte enrichment methods usually need to be conducted in the laboratory with professional equipment and skilled operators. They are therefore not suitable to handle the portable and on-site monitoring applications.

In recent years, the microfluidic technology has opened up great opportunity in developing miniature or micro fluidic devices. In addition to the benefit of portability of reduced sizes and portability, the time and reagent consumptions during the analysis process can be significantly reduced due to the scale effect. As the result, cost of the detection can be significantly reduced. In addition, the microfluidic devices can help to improve the detection sensitivity. In comparison, the conventional methods require the researchers to do the sampling first, then transport the sample to the laboratory for analysis. This means longer test time and laborious work, in addition to the risk of contamination during the transport and storage. A portable equipment with high sensitivity and selectivity has unique advantages and is therefore highly desired [45]. Many research efforts have been made to explore possibility to adopt the microfluidic technology. Xu et al proposed a continuous in situ extraction system based on superwettable membrane [46]. Their approach was based on liquid extraction and laser fabrication was used to generate the nanostructures on the membrane. An interesting microfluidic device was presented by Foan et al [45] for fast extraction of PAHs from water. PDMS film was used as the sorbent and an external pump was used to driven the sample and reagents. Lafleur et al [23] tried to mount the C18 column into a lab-on-a-CD system and utilize the centrifugal force to drive the sample through the sorbent. No valving system was involved and the elution procedure was left
out. Absorption and fluorescence intensity of the sorbent were measured by a spectrometer to analyze concentration of the PAHs.

This chapter reports a novel centrifugal microfluidic platform with mechanical pinch valves for detecting trace amount of oil pollution in water. The platform was prototyped using 3D printing technology and experimentally tested. SPE method is adopted and different stationary sorbent were compared. With the help of 3D printing technology, the centrifugal platform in this chapter is a truly three-dimensional one. Even the sorbent can be 3D printed. The mechanical pinch-valves are highly reliable. The motions of fluid samples and reagents can be simply controlled by manipulating the rotating speed of the platform. The SPE process is performed automatically.

2.2. A centrifugal microfluidic platform for SPE

2.2.1. Design of the centrifugal microfluidic platform

The oil enrichment system was designed based on the conventional solid phase extraction (SPE) principle. The technology uses solid materials to absorb the desired compounds of interest from the liquid sample, and then release them to the desorption solvent. It is therefore the technology used in the presented design. The operational principle of the centrifugal system is schematically demonstrated in Figure 2.1. The centrifugal system consists of two major sections, one is the valving disc for valving actuation [47], and the other one is the fluidic cartridge containing all the fluidic channels and chambers to manipulate the fluidic sample and fluorescence detection. The microfluidic cartridge and the valving disc are mounted on the same shaft, which is by a DC motor. The bottom valving system is separated from the microfluidic cartridge. Therefore, the microfluidic chip can be replaced easily and disposable, while the mechanical valving disc is a permanent component of the system [47]. SPE method was used to extract the oil molecules from the aqueous sample into an organic solvent, and then measured. The enriched sample was then collected in the detection chamber for measurement. For the sake of dynamic balance, two testing units are symmetrically located with respect to the axis.
In each of the two units, there are three chambers (marked as a, b, and c respectively in Figure 2.1) near the center of the disc, the largest one is used to load the oil containing water sample, with the other two are designed to preload the pretreating buffer and organic eluent respectively. Each of the three chambers has a micro-hole at its bottom. The micro-hole is connected to the inlet of the extraction unit (sorbent chamber). The centrifugal force helps to transfer the aqueous phase sample and reagents to the microfluidic network via the holes during rotation. The specially-designed valving disc helps to synchronize the releasing sequentially the reagents and their flow direction. When oil enrichment is completed, the system stops spinning and the enriched sample is collected in detection chamber. As shown in Figure 1, an external UV light is introduced through the window designed on the side wall of the chamber. The excitation UV light is introduced through the window. A receiving fiber is positioned in perpendicular to the excitation light to avoid cross-contamination of measurement signal. The fluorescence light signal is collected and transferred to a spectrometer for measurement.

The working principle of the fluidic cartridge is schematically shown in Figure 2.2 for better explanation. Pretreating buffer, organic eluting solvent, and the stationary sorbent
are prefilled in chambers $a$, $b$, and $c$, respectively. Chamber $e$ is designed as the fluorescence detection region. As shown in Figure 2.2(a), valves $V_2$, $V_3$, and $V_5$ are normally-closed ones, while valves $V_1$ and $V_4$ are the normally-open. As the operation starts, the pretreating buffer solution goes through valve $V_1$ first to condition the sorbent in the chamber $d$, then driven by centrifugal force to flow through the waste outlet as shown in Figure 2.2(b). Valve $V_2$ is then opened and the sample goes through the sorbent in chamber $d$ and flows out as well. In this process, oil molecules in sample are extracted by the stationary sorbent as shown in Figure 2.2(c). Valve $V_4$ is then closed, and valve $V_3$ and $V_5$ are opened. Organic solvent in chamber $c$ is released. As shown in Figure 2.2(d), the solvent fluid flows through chamber $d$ and is collected in chamber $e$. As a result, oil molecules adhered on sorbent are washed off and concentrated in the eluting solvent in chamber $e$ and ready for fluorescence detection.

2.2.2. Design of the centrifugal microfluidic platform

A comprehensive valving disc was designed and applied on the centrifugal microfluidic platform. As shown in Figure 2, $V_1$ was a capillary valve as commonly used in Lab-on-CD systems [48, 49]. The rest of the valves are mechanical pinch-valves, whose operations were independent of the sizes or surface properties of the flow channels. The upper microfluidic cartridge and a slide-based valving disc at bottom were fixed together on a rotating shaft, which was driven by a DC motor.
Figure 2.3 shows the design of the slide-based valving disc. The operational principle of the mechanical pinch-valves is shown as Figure 2.4. By using 3D printing technology, the complex three-dimensional structures can be easily fabricated. At the bottom of the microfluidic cartridge, there were several special designed valving chambers. And a spring plunger was placed beneath each valving chamber. When the spring plunger pushes against the covering silicone membrane of the valving chamber, a deflection is created to block the fluid flowing through the valving chamber, as shown in Figure 2.4(a). The bottom end of the spring plunger had a rotatable ball. The rotatable ball of the spring plunger maintains contact with the upper surface of slide of the valving chip. The valving chip is attached on top of a sliding weight. The grooves on the valving chip were specially design and fabricated, or “mechanically programmed”. As the platform rotates, the centrifugal force drives the sliding weight and valving chip to move outward. The grooves on the valving chip are designed so that at a given rotating speed, the spring plunger would drop into a particular groove on the valving chip. This then causes the spring plunger to stop compressing the membrane above it, and therefore open the valve, as shown in Figure 2.4(b). As the rotating speed continuously increases, the plunger is driven out of the groove, this would cause the spring plunger to compress the valve membrane to close it again as shown in Figure 2.4(c). When the rotational speed is reduced, the centrifugal force is also decreased, and the spring force then helps to restore the weight and the valving chip to its original position. Therefore, by carefully designing the grooves on top of the valving chip, multiple valves can be “mechanically-programmed” to close/open sequentially. A detailed discussion of the design and test of the mechanical valving system had been reported earlier [47].

The grooves on the valving chip are 3 mm × 2 mm (width × depth). The distances between the bottom of the plungers of normally closed valves V₂, V₃, and V₅ and their corresponding grooves are 4 mm, 7 mm, and 7 mm respectively. The plunger of the normally opened valve V₄ dropped in its corresponding groove at the normal state. And its move-out distance was designed to be 4 mm. The diameter and the height of the spring ball plungers
are 3 mm and 6 mm respectively. The weight, a 3D printed cubic box filled with a lead cube, is about 17 grams. The stiffness of the spring is about 1450 N/m. The surface of the valve chip and the valve guide slot were polished with fine grit sandpaper and sprayed with dry lubricant (16-TDL, B’laster) to reduce friction.

![Image](image1.png)

Figure 2.3. Design of the slide-based valving disc.

![Image](image2.png)

Figure 2.4. Operational principle of the “mechanically programed” valves in the centrifugal platform: (a) valve is closed (stationary status or low rotating speed); (b) valve is opened (rotating speed increased and weight moved for a certain distance); (c) valve is closed again (rotating speed is further increased and the spring plunger moves outside of the groove).

2.2.3. Assembly of the microfluidic cartridge

The schematic diagrams in Figure 2.5 shows the assembly of the microfluidic cartridge. The PLA microfluidic structure has an overall dimensional size of 124 mm by 50 mm by 12 mm. The cross section of the channel of the capillary valve $V_1$ has a width of 600 µm and height of 800 µm. The top surfaces of the PLA microfluidic structure were carefully
polished. Then stationary sorbent was loaded into the chamber d. The top and the side window of the PLA microfluidic structure were sealed using transparent polypropylene adhesive tape (Scotch tape, 3M) to prevent any fluid leakage. Vent holes were punched on the tape. Double sided pressure-sensitive adhesive (PSA) tape (3M 442KW) was used to bond the bottom of the PLA structure and the silicone elastic film. Holes were drilled on the PSA tape at the valving chamber positions in advance. The thickness of the PSA tape and silicone film were 100 µm and 0.5 mm, respectively.

![Figure 2.5. Schematic diagram of assembling the microfluidic cartridge.](image)

2.2.4. Construction of the system

Most of the components in the system, such as the microfluidic cartridge, valving chip, valve holder, supporting cover, waste collector, etc., were 3D printed. They were modelled by Solidworks (Dassault Systems SolidWorks Corp, USA), and fabricated by a dual extruder FDM printer (Ultimaker 3, USA). Polylactic acid (PLA) was chosen as the construction material for 3D printing. The water-soluble material polyvinyl alcohol (PVA) was used as the supporting material so that some suspending structures such as the valving chambers shown in Figure 2.4 could be fabricated precisely. The 3D printing of the microfluidic cartridge took about 12 hours. The cartridge was then immersed in water for 24 hours to clean up the residuals of PVA.

Figure 2.6 shows several photo images of the prototype system. Figure 2.6(a) shows image of the assembled prototype. A DC servo motor was used to drive the shaft. Both the
camera and the motor were controlled using a personal computer, which can be replaced in future with an Arduino NANO for integration purpose. As shown in Figure 2.6(b), stationary phase sorbent was pre-sealed in the sorbent chamber \(d\) in the microfluidic cartridge. In Figure 2.6(c), the weight of the valving system was installed in a slot of the holder, which was designed as a guide rail. The valving chip was mounted on the top of the weight. As shown in Figure 2.6(d), a cover with several holes was aligned and mounted on the valve holder with the spring plungers inserted in them. This cover helps support the plungers and permit them to move vertically. The microfluidic cartridge and the valving system were aligned and tightly mounted on a shaft driven by a servo motor.

Figure 2.6. Photo images of the prototype of centrifugal microfluidic platform. (a) image of the assembled system; (b) a close-in image showing the detailed design of the cartridge; (c) the weight and valve chip; (d) plungers.

2.3. Experiment results and discussion

2.3.1. Materials and fabrication

C18 powders, activated charcoal granular, and porous 3D printing filament were adopted as the stationary sorbent respectively in the SPE experiments and the results were compared. Table 2.1 shows detailed information of the three sorbents used in the experiments for comparison purpose.
### Table 2.1. The sorbents used in the experiment.

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>State</th>
<th>Size</th>
<th>Printability</th>
<th>Cost</th>
<th>Vendor and Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18</td>
<td>Powder</td>
<td>40-60 µm</td>
<td>No</td>
<td>High</td>
<td>Thermo Scientific, HyperSep</td>
</tr>
<tr>
<td>Activated Charcoal</td>
<td>granular</td>
<td>250-850 µm</td>
<td>No</td>
<td>Low</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Porous Filament</td>
<td>Filament</td>
<td>1.75 mm</td>
<td>Yes</td>
<td>Low</td>
<td>PORO-LAY, LAYFELT</td>
</tr>
</tbody>
</table>

In chemical analysing experiments, such as high-performance liquid chromatography (HPLC), C18 column is widely used as the stationary phase sorbent due to its outstanding absorption capability of the non-polar compounds. The number 18 in C18 stands for the length of the functional alkyl chain bonded on the silica powder. In the experiments, 60 mg of C18 powders was loaded in the sorbent chamber as shown in Figure 2.7. And a small block of filter paper was inserted into the outlet of the sorbent chamber to prevent washing away the C18 powders by the fluid flow. Figure 2.8(a) shows the SEM image of the C18 powder sealed in the sorbent chamber.

![Figure 2.7. Microfluidic cartridge with different sorbents.](image)

Activated charcoal is another stationary sorbent widely used in SPE but usually much cheaper than C18. Activated charcoal was also tested. In the experiments, 25 mg of activated charcoal granular of 20-60 mesh (particle size 250-850 µm) was loaded in the sorbent chamber. A small piece of filter paper was also inserted into the outlet of the
chamber in advance to prevent the sorbent being wash away. Figure 2.8(b) shows the SEM image of the activated charcoal granular sealed in the sorbent chamber.

Figure 2.8. SEM images of different sorbents. (a) C18 powder; (b) activated charcoal granular; (c) an overall view of 3D printed sorbent; (d) a zoomed in view of the 3D printed sorbent.

A porous 3D printing filament (Lay-Felt, 1.75 mm) was also tried as stationary sorbent. This filament was mixed with water soluble PVA and rubber elastomeric polymer components and could be used in Fused Deposition Model (FDM) printer. When immersed in water, the PVA component is dissolved while the rubber elastomeric polymer remains. Therefore, a porous polymer structure can be obtained in this way. Researchers tried to
use Lay-Felt to 3D print membrane structures [50, 51]. It is also feasible to use this material as the stationary sorbent to catch the oil molecules since the rubber polymer is also a non-polar material. A Lay-Felt sorbent block (4 mm by 4 mm by 6 mm) was fabricated with a FDM 3D printer (Flashforge Creator Pro, China). The extrusion temperature was set at 200 °C and the printing bed temperature was 50 °C. The layer thickness was 0.18 mm and extruder nozzle diameter was 0.4 mm. It took about 15 min to finish the fabrication. Then the sorbent block was immersed in water for more than 24 hours and dried in room temperature. It was then inserted into the sorbent chamber and tested. Figure 2.8(c) and (d) show the SEM images of the 3D printed porous polymer (after washing) sealed in the sorbent chamber. Figure 2.8(c) is an overall view of the surface of the 3D printed polymer. Figure 2.8(d) is a zoomed in images of the 3D printed porous sorbent. It can be observed from the SEM images that the 3D printed porous polymer shows a lot of cavities and pores of micron and submicron level after washing, which significantly increased the roughness of the surface of 3D printed layers and strands, and lead to a higher specific surface area. However, its specific surface area is still less than C18 or activated charcoal.

2.3.2. Oil enrichment experiment

The movements of the water-oil mixture sample, pre-treatment buffer, and the organic eluent during the experiment can be explained as follows by referring to the photo image in Figure 2.7. In the SPE experiments, 0.6 ml methanol was first loaded in chamber $a$ as the pre-treating buffer to condition the stationary sorbent. Sample fluid of 10 ppm (parts per million) oil-water mixture was prepared using Pennsylvania light crude oil (ONTA, Canada) and distilled water (Chemworld, CWDISW-32Z). Six millilitre of fluid sample was then loaded in chamber $b$. 0.6 ml Nonane (Sigma-Aldrich, 296821) was loaded in chamber $c$ as the organic eluent to desorb the crude oil molecules attached to the stationary sorbent. The centrifugal platform was controlled using a personal computer. Firstly, the rotational frequency of the platform was set as 500 revolutions per minute (rpm) for 30 s. At this speed, the methanol in chamber $a$ passed through the capillary valve $V_1$, and was released
to into sorbent chamber $d$, then went out through channel $f$ to the waste collector. As the rotation frequency was increased to 750 rpm and the pinch valve $V_2$ was opened. The platform was kept at this rotation frequency for 5 min to permit the sample in chamber (b) to be completely released and flow through the sorbent. When the rotation frequency was further increased to 950 rpm, the pinch-valves $V_3$ and $V_5$ were opened while the valve $V_4$ was closed. This permits nonane in chamber $c$ to be released to sorbent chamber and desorbed the crude oil molecules, and then collected in the detection chamber $e$. The rotational frequency was kept at 950 rpm for 30 s and then ramped to 1200 rpm and maintained at that frequency for another 30 s to minimize the residual eluent in sorbent chamber. When the centrifugal platform was completely stopped, fluorescence detection was conducted as shown in Figure 2.1.

2.3.3. Results and discussion

A UV light source (Thorlab M365f1) with peak wavelength of 365 nm was adopted as the excitation light source. The fluorescent spectrum of the excited light was measured using an OceanOptics USB4000 Spectrometer. Figure 9 shows the fluorescence intensity before and after SPE process. The blue dashed curve shows the fluorescence intensity of the standard oil-water mixture sample (10 ppm). The green, red and yellow curves represent the fluorescence intensity of eluent (nonane) after SPE with C18 (green), activated charcoal (red), and porous polymer (yellow) as the stationary sorbent respectively.

![Fluorescence intensity before and after SPE process.](image)

Figure 2.9. Fluorescence intensity before and after SPE process.
From the experimental results in Figure 2.9, it can be observed that the fluorescence intensities using different SPE materials were all significantly increased after SPE with the centrifugal microfluidic-platform. The fluorescence intensity after SPE with using C18 as sorbent material was about three times of that for the original sample. The activated charcoal has a similar extraction efficiency as C18 though the fluorescent spectrum is slightly different, with higher intensities between 400 nm to 480 nm. A much higher enrichment efficiency was achieved when the 3D printed porous polymer was used as the stationary sorbent. The fluorescence intensity increased more than 6 times. In these experiments, waste fluids were all collected in the waste collector after SPE. They were also tested with the same UV light source and spectrometer and no measurable fluorescence were detected. This means all the crude oil molecules in the mixing sample were adsorbed by the stationary sorbent in each experiment. Therefore, the concentration difference of oil molecules in the eluent is primarily the results of different eluting efficiencies. In the experiment, the eluent was released while the centrifugal platform working at a relatively high rotating frequency of 950 rpm. As a result, the centrifugal force was relatively large at this moment and made the eluent went through the sorbent quickly. C18 and activated charcoal used in the experiment had larger specific surface areas and might be more adhesive to oil molecules, so more eluent and more eluting time were required to minimize the residual oil molecules in the sorbent. On the other hand, the oil molecules might be relatively less adhesive to the porous polymer so the eluent could bring them out more easily. The reason of the slightly difference between C18 and activated charcoal from 400 nm to 480 nm may be more complicated. As mentioned previously, the fluorescence of the crude oil was caused by PAHs, a group of organic compounds with two or more aromatic rings. The sorbent adhesion to each PAH molecule are different and each PAH molecule has its own fluorescence spectrum. Therefore, the fluorescence intensity curves under different conditions (with different sorbent) are not identical.
As shown in Figure 2.9, the 3D printed porous polymer showed a very promising performance with the 10 ppm oil/water mixing sample. To further study the performances of using 3D printed porous polymer as stationary sorbent, the experiments were conducted using testing samples with concentrations of 2 ppm and 5 ppm respectively. Figure 2.10 shows the experimental results of the fluorescence intensities before and after SPE process. The fluorescence intensities of the eluent after SPE were about 5-6 times of the fluorescence intensities of the original samples. Figure 2.11 shows the comparison of integrated fluorescence intensities before and after SPE process. To avoid affection of the excitation light, fluorescence between 410 nm to 650 nm were integrated and compared.

Figure 2.10. Fluorescence intensity before and after SPE process with 2 ppm testing sample (a) and 5 ppm testing sample (b).

Figure 2.11. Comparison of integrated fluorescence intensities before and after SPE.
In the experiments, the sorbents were loaded into the sorbent chamber manually. However, it should be noted that this can be avoided when the 3D printable porous polymer was used as the sorbent with one more extruder installed in the 3D printer. Multiple materials can be fabricated with more advanced 3D printer.

2.4. Conclusions

This chapter presented a novel centrifugal microfluidic platform for enrichment and fluorescence detection of spilled-oil in water. The platform was based on solid phase extraction using centrifugal actuation. Mechanical pinch-valves were applied to control the reagent flows. The valving system was controlled by manipulating the rotation speed of the centrifugal platform. The prototype of the proposed system was 3D printed with PLA material. Water soluble PVA was used as supporting material to help print the complex microfluidic structure precisely. The platform was tested with 10ppm standard oil-water sample. In the experiment, C18, activated charcoal, and 3D printed porous polymer were used as stationary sorbent respectively and compared. The 3D printed porous polymer was proved to have the best SPE performances with about six times of enrichment efficiency. The experiment results demonstrated functionality of the prototype lab-on-CD system. And the 3D printable porous polymer showed a very promising performance in the experiment. This centrifugal device can potentially be used as a portable on-situ spilled oil enrichment and detection device. Other than crude oil, this platform can be also used for other pollutants in water for environmental protection applications.
Chapter 3. A novel gravity valve and its application in a 3D printed centrifugal fluidic-system for SPE of PAHs in water

3.1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds consisting of two or more aromatic rings. These nonpolar molecules widely exist in fossil fuels like coal and crude oil. PAHs can be produced during the incomplete combustion of organic matter, such as coal, oil, wood, and et al. Reports show that PAHs can accumulate in human body and are toxic and carcinogenic to living organisms. Usually, more aromatic rings in PAHs mean lower acute toxicity but higher carcinogenicity. In addition, PAHs may also cause genetic mutation. Due to the extremely long degradation period, PAHs are viewed as persistent organic pollutants (POPs) [52, 53, 54, 55, 56]. As the industrialization spreads across the world, more pollutants containing PAHs are produced, and therefore caused more environmental pollution. Because of their severe health consequences, it has become very important to accurately monitor and control PAHs in recent years. The World Health Organization (WHO) has also set up the standards to limit the concentration level of PAHs in water, air, soil, and food [57].

PAHs are commonly detected with spectroscopic analysis technologies. When exited by UV light, each of the PAHs emits fluorescence in its own fluorescence spectrum. For high precision detection of PAHs in the environment, pre-enrichment of the target molecules is the primary step. Researchers have made tremendous efforts to find good methods to extract the PAHs particles for sample enrichments. In a typical solid phase extraction (SPE) process, analyte is first adsorbed from the aqueous sample to the selected sorbent, and then desorbed to eluent to extract and enrich PAHs from aqueous sample [58, 59] for enhanced detection sensitivity. However, traditional SPE method requires professional handling and complicated equipment, and usually conducted in laboratories by skilled operators.

Many efforts have been made to develop a portable automated SPE system for on-site monitoring application. Kira et al [60] reported a portable sampling device consisting of a SPE cartridge and an external pump. It could be used to continuously sample PAHs in environment water. Heub et al [61] presented an automated portable SPE platform for immunoassay application. This platform was an integration of bottles and tubes required in SPE as well as an external pump. Foan et al [45] developed a microfluidic chip for PAHs extraction with PDMS film as the sorbent. The sample and reagents were delivered using an external syringe pump.

The first centrifugal fluidic platform was developed in 1969 [1]. Centrifugal fluidic systems have been widely used in many biological, chemical, and medical applications because of its unique advantages of eliminating external pumping function and simplified structures [2, 62]. The processed volume on the platform can vary from milliliters to nanoliters with almost no residual. With proper design of the channels and other fluidic components on the platform and manipulations of the rotation speed, sequential control of valves and flows can be achieved [2, 47]. Because of these attractive advantages, the technology has found applications in fields like nucleic acid analysis, immunoassays, clinical chemistry, cell handling, water analysis, and et al [2].

Attempts had also been made to combine SPE on centrifugal platforms. Hoffmann et al [63] presented a lab-on-a-chip cartridge used on centrifugal platform for DNA extraction. The system had no valves and the reagents must be preloaded in glass ampoules and released manually. Lafleur et al [23] reported a µ-CD platform for extraction of anthracene from water. C18 column was embedded and centrifugal force was used to deliver the conditioning buffer and testing sample. No elution procedure was developed and fluorescence was directly detected from the C18 column and reported a detection limit of 1 mg/L (20 ng).

Efficient valving technology is the key to realize automatic SPE on a centrifugal platform. To achieve the best SPE efficiency, the variable speeds of rotation are needed:
1) The aqueous sample of PAHs-water needs to flow through the sorbent chamber in slow speed to have better adsorbing effect;

2) Higher rotational speed is then needed to minimize the residual water in sorbent chamber;

3) Next, the eluent needs to flow through the sorbent chamber slowly (therefore lower rotational speed), so that there is enough time for the eluting process (the last procedure of SPE).

Most of the passive valves (such as capillary valve) used in centrifugal fluidic platforms are the normally closed ones. They are turned to open by simply increasing the spinning frequency to their corresponding burst frequencies [2]. Sequential release of reagents from pressure sensitive stick packs may realize the similar function [64, 65]. In contrast, siphon valves are normally-open during the intermediate static status or low spin frequency status, and will be closed at high speed. However, the slender micro-channel of the siphon valve must be carefully designed and precisely fabricated. So it is difficult to integrate a siphon valve in a 3D printed cartridge. On the other hand, active valves can be either normally closed or normally open. However, the active valves are usually controlled and actuated by external means other than centrifugal force.

In this chapter, a novel gravity valve is designed and used in addition to capillary valves and Coriolis switching valve [3]. Different from the mechanical valves used in presented in the previous chapter, no external actuation valving mechanism is need. The system is therefore dramatically simplified. What’s more, the reported cartridge can fully satisfy the requirements presented previously for automated SPE on a centrifugal platform. By taking the advantages of the 3D printing technology, a new type of gravity valve, Coriolis switching valves, and the traditional capillary valves were integrated in the same centrifugal fluidic cartridge, and printed as a solid piece. The aqueous reagents and samples in the cartridge were simply controlled by the spinning direction and speed of the platform. SPE experiment was conducted with the presented design and anthracene was used as the analyte.
3.2. Designs and fabrication of the valves and the centrifugal fluidic cartridge

3.2.1. Design of the gravity valve

When a centrifugal platform rotates in horizontal plane around a vertical axis, a fluid sample would be subject to three major forces: gravity, centrifugal, and Coriolis forces, in addition to flow resistance. When a fluid sample flows across a chamber in the centrifugal platform, the flow resistance of air can be assumed negligible. The centrifugal, gravity, and Coriolis forces are perpendicular each other in vertical and horizontal direction respectively. When the platform rotates at high speed, the centrifugal force becomes dominant so the aqueous sample is driven to flow outward. On the other hand, when the rotational speed of platform is low, or stops rotating, the gravity force becomes dominant, and the effect of the centrifugal force becomes less important in the motion of the fluid sample. The gravity force then drives the aqueous sample to flow downward. As the result, a special designed 3D structure and programmed rotational sequence can be used to control different samples be released to the targeted chambers sequentially. Based on this theory, a novel gravity valve was designed and fabricated in the 3D microfluidic SPE cartridge. Figure 3.1 shows the working principle of the gravity valve. As shown in Figure 3.1(A), sample 1 and sample 2 were preloaded in chamber a and chamber c respectively. When the cartridge was positioned horizontally and rotates around the vertical axis, the centrifugal force drives sample 1 to flow along the slope into the transitional chamber b, and also drives the sample 2 to the collecting chamber, as shown in Figure 3.1(B). As far as the rotational speed is high enough so that the centrifugal force helps to keep the sample 1 inside the transitional chamber. As shown in Figure 3.1(C), when the cartridge stopped rotating, gravity force then drives sample 1 to flow downward through the ”gravity valve” to enter chamber c. When the platform started rotating again, centrifugal force would drive sample 1 to the collecting chamber as shown in Figure 3.1(D). The experiments have proved that this gravity valve was a very reliable. Compared with capillary valves, which can only be sequentially opened from low frequency to high frequency, the operation of gravity valve is
more flexible. It is very suitable for the applications that require sample 2 being released firstly at a relative high rotating speed and then sample 1 being released at a relative low rotating speed.

3.2.2. Coriolis switching valve

In addition to the widely used centrifugal force, the Coriolis force was also used in flow control of a centrifugal system. The Coriolis force is an inertial or fictitious force that acts on an object that has a relative motion in a rotating platform with respect to an inertial frame. The Coriolis force $F_{co}$ acted on a droplet of fluid in a rotating platform can therefore be calculated by equation (1.2). The Coriolis force acts perpendicular to both angular velocity and the centrifugal force and generates secondary flow phenomena [3]. As a result, a passive flow switching valve controlled by the rotating direction can be built in the centrifugal system [3, 66]. Figure 3.2 shows the working principle of the Coriolis switching valve as used in the presented design of the system. If the reference platform (the cartridge) rotates clockwise, the Coriolis force causes the liquid sample to flow into
the right-side chamber in the cartridge as shown in Figure 3.2. If the reference platform rotates counter-clockwise, the Coriolis force forces the liquid sample to enter the left-side chamber in the cartridge.

![Figure 3.2. Working principle of the Coriolis switching valve.](image)

3.2.3. Design of the truly three-dimensional SPE cartridge

Taking advantage of the gravity valve and switching valve as discussed in the foregoing sections as well as capillary valves, automated Solid Phase Extraction (SPE) can be realized on the centrifugal platform as shown in Figure 3.3. Chambers a, b, and c in Figure 3.3 correspond to chamber a, b, and c in Figure 3.1, which graphically demonstrated the operation of the gravity valve system. Eluent, conditioning buffer, and testing sample are preloaded into chamber a, c, and d respectively as shown in Figure 3.3(A). When the cartridge spins counter-clockwise at low speed as shown in Figure 3.3(B), the eluent flows into transitional chamber b. Meanwhile, conditioning buffer flows through chamber e to activate the sorbent and comes out from the waste outlet under centrifugal force and the Coriolis force. The sample will not be released because the capillary valve connected to the sample chamber has higher burst frequency. If the spinning speed reached the burst frequency of the sample, the sample fluid flows through chamber e and comes out from the waste outlet to permit the PAHs in the sample absorbed in the sorbent as shown in Figure 3.3(C). The platform was then brought to completely stop, and the eluent in chamber b flows into chamber c due to gravity as shown in Figure 3.3(D).
the platform then spins clockwise, the eluent is driven to flow through the sorbent chamber and collected in detection chamber f due to the centrifugal force and the Coriolis force. Finally, the platform stops to rotate so that the fluorescence detection can be conducted with perpendicular fibers as shown in Figure 3.3(F).

Figure 3.3. Schematic diagrams showing the working principle of automated Solid Phase Extraction (SPE) on the centrifugal platform.

On account of the trace amount and uneven distribution of analyte in environment water, sufficient sample is required to with our design. However, too much sample and reagents will increase the load and size of the centrifugal platform as well as the testing time. A few preliminary tests were made and then the sample and eluent volume for the stereotype were set as 10 mL and 1 mL. In view of this, the microfluidic cartridge was designed to have a physical dimension of 150 mm × 50 mm × 20 mm (length ×
width × height). Figure 3.4(A) shows an overall view of the three-dimensional design for microfluidic SPE cartridge, created using Solidworks (Dassault Systems SolidWorks Corp, USA). There were two working units on one cartridge, and they were axial symmetric with respect to the rotational axis. Figure 3.4(B) is a lateral cross-section view of the cartridge showing the 3D structure of the gravity valve. Figure 3.4(C) is a horizontal cross section view of the cartridge showing the designed capillary valves connected to the sorbent chamber. Solid sorbent, conditioning buffer, testing sample, and eluent are preloaded in corresponding chambers. Transitional chamber for gravity valve was designed as quarter-funnel-shaped to minimize the residual. Orthogonal windows were designed on the sidewall of the detection chamber for fluorescence detection. There were three capillary valves in the cartridge. Capillary valve 1, which connects eluent chamber a and transitional chamber b, was designed to have a cross-section of 1 mm × 1 mm. Capillary valve 2, which connects conditioning buffer chamber c and sorbent chamber e, was designed to have a cross-section of 800 µm × 800 µm, relatively large size to achieve a low burst frequency. Reagents can therefore flow through these two valves when the platform rotated at a very low speed. Capillary valve 3, which connects sample chamber d and sorbent chamber e, was designed to have a cross-section of 250 µm × 250 µm. These numbers were chosen based on results of many experiments with the 3D printer in our laboratory. In the practice of FDM 3D printing manufacturing, the cross-section of flow channel at this scale tends to be irregular and the actual sizes tends to be much smaller than the designed value. Therefore, it had a relatively higher burst frequency than the designed channel. On the other hand, the microchannel might be blocked during the printing process if the designed size was too small. A Coriolis switching valve was designed at the outer end of sorbent chamber e. The outlet of the Coriolis switching valve was designed as 600 µm × 600 µm. The liquid sample can be controlled to either enter the left-side waste chamber or right-side collection chamber for fluorescence detection by reversing the rotational direction of the platform.
Figure 3.4. Design of the 3D microfluidic cartridge. (A) An overall view of the 3D microfluidic cartridge; (B) Lateral cross section view of the 3D cartridge; (C) Horizontal cross section view of the 3D cartridge.

3.2.4. Fabrication and Set up of the system

PLA (Polylactic Acid) was used as the structural material of the microfluidic cartridge. The cartridge was fabricated using a Fused Deposition Modelling (FDM) 3D printer (Ultimaker 3). The nozzle of the print core was 0.4 mm. The 3D printing process was configured with 0.1 mm layer height and 10% infill density. The printing temperature and build plate temperature were set as 200 °C and 60 °C respectively. It took about 13 hours to 3D print one cartridge using the low-cost printer. And the printed cartridge was about 53 g. Figure 3.5 shows the additive manufacturing flow chart, which helps a better understanding of the three-dimensional structure of the system.

Figure 6(A) shows the schematic design diagram of one working unit of the SPE centrifugal cartridge. Figure 6(B) shows a top-view photo image of one working unit of the
3D printed prototype cartridge. Figure 6(C) shows a photo image of the overall view of the fabricated centrifugal cartridge with two working units, which can be compared with the schematic design shown in Figure 4(A). Figure 6(D) shows a photo image of the lateral cross-sectional view of the centrifugal cartridge, which can be compared with the schematic diagram shown in Figure 4(B). The photo in Figure 6(E) shows the horizontal cross-sectional view of the 3D printed cartridge, which can be compared to the schematic design diagram as shown in Figure 4(C).

Physical dimensions of three printed the cartridges were carefully measured and compared with the designed number. Their overall dimensions were slightly larger than the design but the errors were less than 0.5mm. And the processing errors of each chambers were all less than 0.3 mm. These errors did not show any significant impact on their functions. It is worth to mention that the cartridge was 3D printed with white PLA for the sake of visual demonstration and fluidic functionality test. In the experiment discussed next section, the cartridge was 3D-printed with black PLA to minimize the reflection of the excitation light and other noises for better measurement sensitivity in fluorescence detection.

The 3D printed cartridge was then polished with sand paper and sealed with transparent polypropylene adhesive tape (Scotch tape, 3M) to prevent flow leakage. Air vents were punched on the covering tape. The prototype device was then mounted on the DC servo-motor controlled with a laptop computer and tested. A high-speed camera was installed above the device to monitor the flows of the samples and reagents in real time. Figure 3.7 shows a photo image of the workbench of the centrifugal platform with 3D microfluidic cartridge mounted.

3.3. Experimental results and discussions

3.3.1. Functionality test

Dimensional measurement and preliminary experiments were implemented to check the functionality of the presented design and determine the suitable operational parameters
Figure 3.5. Flow chart of additive manufacturing process for the 3D microfluidic cartridge.

Figure 3.6. The 3D printed prototype of the fluidic cartridge. (A) The graphical design of one working unit; (B) the photograph of one 3D printed working unit; (C) overall view of the 3D printed cartridge; (D) Lateral cross section view of the 3D printed cartridge; (E) Horizontal cross section view of the 3D printed cartridge.
such as the rotational speeds (burst frequency for capillary valves) and time duration needed in each speed for synchronized releases of the fluid sample and reagents.

Fused Deposition Modeling (FDM) is a low-cost 3D printing technology that can be used to construct complex 3D structures. However, it has the disadvantage that the printing resolution is lower compared with conventional microfabrication techniques based on photolithography. As mentioned previously, the actual sizes of the 3D printed microchannels were usually smaller than the designed values. And the cross-section of the fabricated channel also tends to be more irregular instead of the ideal rectangular one designed as the printing size decreases. To find out how the actual size of the fabricated channel compared with the designed one, flow channels with different sizes were 3D printed with identical
configuration with the cartridge. The cross-sectional area of the flow channel was designed to range from 200 \( \mu m \times 200 \mu m \) to 1000 \( \mu m \times 1000 \mu m \). Five sets of channels were fabricated and measured using a microscope. Figure 3.8 shows a microchannel with the designed cross-section of 250 \( \mu m \times 250 \mu m \). Figure 3.9 shows the relation between sizes of the fabricated and the designed micro channels. The labeled numbers in the diagram are the mean values of 5 sets channels measured. The error bars show 90% confidence intervals of the printed sizes. Table 3.1 shows more detail about the mean value and the 90% confidence interval of the printed micro channels.

The burst pressure of the capillary valve can be calculated using the following formula [67]:

Table 3.1. Mean value and the 90% confidence interval of the printed micro channels.

<table>
<thead>
<tr>
<th>Designed size (( \mu m ))</th>
<th>Printed size</th>
<th>Width (( \mu m ))</th>
<th>Width 90% confidence Interval</th>
<th>Height (( \mu m ))</th>
<th>Height 90% confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>Width</td>
<td>55</td>
<td>55 ± 9</td>
<td>92</td>
<td>92 ± 27</td>
</tr>
<tr>
<td>250</td>
<td>Width</td>
<td>104</td>
<td>104 ± 21</td>
<td>187</td>
<td>187 ± 22</td>
</tr>
<tr>
<td>300</td>
<td>Width</td>
<td>168</td>
<td>168 ± 8</td>
<td>222</td>
<td>221 ± 13</td>
</tr>
<tr>
<td>350</td>
<td>Width</td>
<td>243</td>
<td>242 ± 19</td>
<td>304</td>
<td>303 ± 17</td>
</tr>
<tr>
<td>400</td>
<td>Width</td>
<td>295</td>
<td>294 ± 14</td>
<td>305</td>
<td>305 ± 8</td>
</tr>
<tr>
<td>450</td>
<td>Width</td>
<td>344</td>
<td>344 ± 11</td>
<td>382</td>
<td>381 ± 21</td>
</tr>
<tr>
<td>500</td>
<td>Width</td>
<td>361</td>
<td>361 ± 24</td>
<td>372</td>
<td>372 ± 12</td>
</tr>
<tr>
<td>600</td>
<td>Width</td>
<td>398</td>
<td>397 ± 35</td>
<td>437</td>
<td>437 ± 20</td>
</tr>
<tr>
<td>800</td>
<td>Width</td>
<td>630</td>
<td>630 ± 22</td>
<td>632</td>
<td>632 ± 20</td>
</tr>
<tr>
<td>1000</td>
<td>Width</td>
<td>820</td>
<td>820 ± 38</td>
<td>808</td>
<td>807 ± 34</td>
</tr>
</tbody>
</table>
\[ P = \frac{2\gamma_{la}}{w} \left[ -\frac{w}{h} \cos \theta_c - (\theta_c + \beta) \right] \]  \hspace{1cm} (3.1)

where \( \gamma_{la} \) is the liquid surface energy in contact with air (72 mN/m for water at room temperature), \( w \) and \( h \) are the width and height of the channel, \( \theta_c \) is the contact angle of liquid on solid (about 80 degree for water with PLA [68]), \( \beta \) is the opening angle of the channel expansion (90° for the presented design) [67]. As the burst pressure is obtained, the burst frequency can be further calculated with the following equation:

\[ P = \rho \omega^2 (\Delta r) r. \]  \hspace{1cm} (3.2)

where \( P \) is the pressure, \( \rho \) is the mass density of the liquid, \( \omega \) is the rotational speed of the centrifugal cartridge (rad/s), \( \Delta r \) is the difference between the top and bottom of the liquid levels with respect to the rotation center, \( r \) is the average distance of the liquid to the rotation center [67].

The capillary valve connecting sample chamber and sorbent chamber (capillary valve 3) was designed to have a cross section of 250 \( \mu \)m \( \times \) 250 \( \mu \)m while showing a measured cross section of 104 \( \mu \)m \( \times \) 187 \( \mu \)m (width \( \times \) height) in average. The calculated burst frequencies with 1 mL and 10 mL water loaded in the sample chamber were 730 rpm and 403 rpm. Experiments were implemented to find out the real burst frequency. Five cartridges were fabricated and tested. The measured burst frequencies with 1 mL and 10 mL water loaded in the sample chamber were 652 rpm and 384 rpm in average. And the standard deviation were 38 rpm and 25 rpm. The measured burst frequencies were slightly smaller than the calculated values.

The 3D fluidic cartridge was tested on the centrifugal platform. For easy observation, fluids with different colors are used in the tests. The pink, green, and blue liquids represent the organic eluent (nonane), pretreating buffer (methanol), and testing sample (water),
respectively. Figure 3.10 shows six photographs demonstrating the operation of the system and the releasing sequence of the fluids with different colors from respective chambers.

At the beginning of the test, the pink, green, and blue liquids (nonane, methonal, and water, 1mL each) were preloaded into chamber $a$, $c$, and $d$, respectively using syringes as shown in Figure 3.10(A). The cartridge rotated in counter-clockwise direction first at 400 rpm as shown in Figure 3.10(B). The eluent (pink) in chamber $a$ flowed into transitional chamber $b$. The pre-treating buffer (green) in chamber $c$ flowed through chamber $e$ and then released from the waste outlet. And the testing sample (blue) stayed in chamber $d$ because its burst frequency was not reached. When the spinning speed was increased to 800 rpm, which reached the burst frequency of the testing sample. The testing sample was then released through capillary valve into chamber $e$ first, then flow through the switching valve into the waste outlet as shown in Figure 3.10(C). When the sample chamber was drained, the spinning speed was further increased to 1200 rpm to minimize the residual as shown in Figure 3.10(D). Next, the spinning speed was decreased to zero to permit the eluent in chamber $b$ flowed into chamber $c$ due to gravity as shown in Figure 3.10(E). Then, the cartridge was driven to rotate clockwise at 500 rpm. The eluent flowed through the sorbent chamber $e$ and turned slightly to the left side and finally collected in detection chamber $f$ as shown in Figure 3.10(F).

Due to the surface tension of the liquid and the surface roughness of the 3D printed structure, the eluent (nonane) did not flow completely to chamber $c$ from the transitional chamber $b$ during the procedure as shown in Fig 3.10(E). Experiment was therefore conducted to find out the volume of residual eluent in the following sequence: 1) 1mL nonane was loaded in chamber $a$ first; 2) the cartridge spun counter-clockwise at 2000 rpm to drive nonane flow to the transitional chamber $b$; 3) the platform stopped and nonane went down to chamber $c$ due to gravity; 4) the cartridge spun counter-clockwise again at 2000 rpm to drive nonane in chamber $c$ to exit through the waste outlet; finally, the platform stopped and the residual nonane was measured by measuring the weight difference of the cartridge.
before and after the experiment. The experiment was repeatedly for 4 times, the average value of the residual was 45 mg with a standard deviation of 10 mg. The density of nonane was 718 kg/m$^3$. As the result, the residual nonane was estimated to be about 63 µL, which was about 6.3% of the total eluent. Therefore, the gravity valve is valid for millilitre level applications. It is also possible to be applied on smaller reagent volume if proper redundant reagent is adopted. Dead volumes of other chambers were measured with the same method. Experiment result shows that the dead volume of the sample chamber $b$ was less than 20 µL, and the dead volumes of the conditioning buffer chamber $c$ and sorbent chamber $e$ were less than 10 µL. The residual water volume in the stationary sorbent (C18 powder, 50 mg) was about 30 µL. Compared to the sample and reagent volumes (10 mL and 1 mL) used in the experiment, the dead volumes wouldn’t impact the experiment result significantly. Based on the dead volume measured above, it can be concluded that the presented
design is valid for applications with millilitre level and sub-millilitre level (several hundred microlitres) reagents involved.

3.3.2. Enrichment experiments of PAHs and discussions

The SPE experiment was conducted to verify the feasibility of using the 3D printed cartridge for extraction and enrichment of PAHs in water. Fifty mg C18 (HyperSep) (Thermo Scientific, USA) silica powder was loaded in the sorbent chamber as the stationary sorbent. A small piece of filter paper (Whatman 1003-055) was inserted at the outer end of the sorbent chamber to prevent the C18 powder being washed away during SPE process. The cartridge was sealed with transparent tape to prevent liquid leakage. Anthracene, one of the PAHs, was used as the analyte and tested. Anthracene (1000 µg/mL in acetone, from NSI Environmental Solution, USA) was first diluted with acetone (1:100) to obtain a solution of 10-5g/ml (10mg/L in acetone). The solution was then further diluted in distilled water with the ratio of 1:1000, 1:2000, and 1:10000, respectively to obtain testing samples with concentrations of 10 µg/L (10 ppb, part per billion), 5 µg/L (5 ppb), and 1 µg/L (1 ppb).

Figure 3.11 shows the programmed rotating frequency and the function of each step, in which counter-clockwise is marked as positive while clockwise as negative. In the experiments, the organic eluent (1 mL nonane), pretreating buffer (1 mL methanol), and testing sample (10 mL) were manually preloaded into chambers a, c, and d respectively using syringes. At the beginning of operation, the cartridge rotated counter-clockwise at 350 rpm and 700 rpm for 10 s and 20 s. During this period, the eluent flowed into transitional chamber b, the pre-treating buffer flowed through the sorbent and activated C18 powder, then released from the waste outlet under the centrifugal force and the Coriolis force. The spinning speed was then increased to 1200 rpm and maintained at this speed for 3 min, then further increased to 1600 rpm and 2000 rpm, and stayed at each speed for 2 min and 1 min respectively. During this period, the testing sample flowed through chamber e, with anthracene (the analyte) adsorbed in the chamber and water exhausted from the waste.
outlet. To minimize the residual water in sorbent, the spinning speed was further increased to 2500 rpm and maintained for 1 min. Then the platform completely stopped. The eluent in transitional chamber flowed into chamber $c$ due to gravity. Then, the cartridge rotated clockwise at 800 rpm for 1 min first, then the speed was further increased to 2000 rpm and 2500 rpm and kept for 30 s respectively to minimize the residual eluent in sorbent. The eluent flowed through sorbent and finally collected in detection chamber $f$. Finally, the platform was brought to complete stop and the fluorescence detection of the eluent in detection chamber $f$ was measured.

Figure 3.11. Programmed rotating frequency and the function of each procedure.

In the fluorescence detection, a UV light source with wavelength of 365 nm (Thorlab M365f1) was used as the excitation source. The fluorescence spectrum was measured and recorded with an OceanOptics Flam-S-XR1-ES Spectrometer. Two optical fibres (Thorlab M41L01, 600 µm, 0.48 NA) were used to introduce the excitation light and collect the fluorescence through the windows on the sidewall of the detection chamber. Fluorescence spectra of the samples before SPE were also tested using the same equipment and configuration for the purpose of comparison.

Samples with 3 different analyte concentrations (1 ppb, 5 ppb, and 10 ppb) were tested, and the test was repeated 3 times for each analyte concentration. Figure 3.12 shows the fluorescence spectra before and after SPE for different concentrations of anthracene in water. Anthracene typically emits fluorescence with wavelengths between 365 nm and 500
Because wavelength of the excitation light used in our design was also predominantly around 365 nm, the fluorescence signal of the sample fluid around 365 nm should be reduced in the measurement. In addition, the spectra contents of the fluorescence of the sample above 460 nm were also found to be very weak and vulnerable to noise contamination. Therefore, to minimize the influence of the excitation light and other possible noise, only the fluorescence contents with wavelengths between 390 nm and 460 nm were utilized in the data analysis. The emission intensities of the samples with different concentrations were normalized for comparison. The curve showing the fluorescence spectrum after SPE was obtained by averaging the experimental results with three repeated tests. The Raman scattering of the solvents (water/nonane) were deducted to make the fluorescence spectra more accurate. From the results presented in Figure 10, it can be observed that the peak of fluorescence intensity of the SPE enriched sample was about 10 times of the original one before SPE. Table 3.2 shows more details of the integrated fluorescence intensities before and after SPE with the prototype device. For the testing sample with 10 ppb analyte, the measured integrated fluorescence enhancement ratio of three repeated tests were found to be 7.74, 7.97, and 6.37, respectively. As a result, the average enhancement ratio was 7.36 with a standard deviation of 0.87. The 90% confidence interval of the real mean enhancement ratio was 7.36 ± 1.47. For the testing sample with 5 ppb analyte, the measured integrated fluorescence enhancement ratio of three repeated tests were 8.27, 9.99, and 10.19, respectively. The average enhancement ratio was 9.48 with a standard deviation of 1.05. The 90% confidence interval of the real mean enhancement ratio was 9.48 ± 1.78. For the testing sample with 1 ppb analyte, the measured integrated fluorescence enhancement ratio of 3 repeated tests were 11.41, 12.34, and 11.67, respectively. The average enhancement ratio was 11.81 with standard deviation of 0.48. The 90% confidence interval of the real mean enhancement ratio was 11.81 ± 0.81.

Figure 3.13 shows the comparison of integrated fluorescence intensities from 390 nm to 460 nm and enhancement ratios of different anthracene concentrations. As the concen-
tration of anthracene increased, the integrated fluorescence intensity of the testing sample showed a roughly linear growth. The red curve shows the average measured enhancement ratio of sample with different analyte concentrations. Error bar shows the 90% confidence interval of the real mean enhancement ratio. It can be found from the graph that sample with lower analyte concentration could result in a higher enhancement ratio.

![Fluorescence spectra before and after SPE with different anthracene concentrations.](image)

Table 3.2. Comparison of integrated fluorescence intensity before and after SPE.

<table>
<thead>
<tr>
<th>Original Sample</th>
<th>10 ppb</th>
<th>5 ppb</th>
<th>1 ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integration time</td>
<td>1000 ms</td>
<td>2000 ms</td>
<td>4000 ms</td>
</tr>
<tr>
<td>Integration range</td>
<td>390 - 460 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescence intensity (sample)</td>
<td>91953</td>
<td>119577</td>
<td>54570</td>
</tr>
<tr>
<td>Normalized intensity (sample)</td>
<td>183907</td>
<td>119577</td>
<td>27285</td>
</tr>
<tr>
<td>Fluorescence intensity (eluent)</td>
<td>712113; 732946; 585305</td>
<td>989150; 1194581; 1218327</td>
<td>622549; 673493; 637077</td>
</tr>
<tr>
<td>Normalized intensity (eluent)</td>
<td>1424226; 1465892; 1170609</td>
<td>989150; 1194581; 1218327</td>
<td>311274; 336746; 318539</td>
</tr>
<tr>
<td>Enhancement ratio</td>
<td>7.74; 7.97; 6.37</td>
<td>8.27; 9.99; 10.19</td>
<td>11.41; 12.34; 11.67</td>
</tr>
<tr>
<td>Average enhancement ratio</td>
<td>7.36</td>
<td>9.48</td>
<td>11.81</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.87</td>
<td>1.05</td>
<td>0.48</td>
</tr>
<tr>
<td>Confidence interval (90%)</td>
<td>1.47</td>
<td>1.78</td>
<td>0.81</td>
</tr>
</tbody>
</table>

The volume ratio of the testing sample and the eluent was 10:1 in our test, which means the theoretical fluorescence enhancement ratio was supposed to be 10 if the fluorescence intensity increased with the fluorophore concentration in perfect and linearity and the SPE efficiency is 100%. However, the fluorescence excitation of anthracene is a complex process and the relation between fluorescence intensity and fluorophore concentration is not exactly linear in practice. On the other hand, due to solvent effect, with the same anthracene concentration, fluorescence detected from nonane would be stronger than that
from distilled water. As the result, the fluorescence intensity enhancement ratio doesn’t directly reflect the analyte recovery ratio. Though the measured fluorescence intensity after SPE could be more than 10 times (for example, 11.81 times for 1ppb sample) of the original sample, anthracene might not be completely recovered in eluent. To estimate the recovery ratio of anthracene, the normalized fluorescence intensity of the eluent after SPE was compared with the fluorescence of spiked solution (anthracene dissolved in nonane). The estimated recovery ratio for 10 ppb, 5 ppb, and 1 ppb sample were about 28%, 42%, and 44%, respectively.

There might be two possible reasons that the analyte was not fully recovered in eluent. Some of anthracene might had been flushed into waste collector, or still remained in sorbent. Experiments were conducted to find the reasons. The same fluorescence test was conducted for the water collected in the waste collector. No detectable fluorescence was found in it, which means there was no more measurable anthracene in the waste-water. Next, additional eluent was loaded in the cartridge and eluting process was repeated. The eluent was collected and tested under UV light. Fluorescence was detected from the eluent. Therefore, it can be concluded that the anthracene in the testing sample was completely extracted by the sorbent in the microfluidic SPE cartridge, but not completely desorbed.
from the sorbent during the eluting process. Residual water in the sorbent powders after testing sample went through might be one of the reason caused insufficient desorption during the elution. In addition, the entire SPE process was finished in 10 min and the eluent process only took 90 s. This could be another reason caused insufficient eluent.

The integrated fluorescence intensity of the eluent after SPE with our design was found to be from 7 to 11 times compared with the original sample depends on the analyte concentration of the original samples. This result means that the fluorescence detection limit was improved for 7 to 11 times compared with original sample when the same optical device was used. Additionally, the proposed centrifugal system could largely reduce the working time and simplify the operational complexity compared with traditional SPE method, which is very important for an on-site monitoring device.

Perylene, another PAHs with 5 aromatic rings, was also tested as the analyte. First, 0.01 g perylene powder (Sigma-Aldrich, P11204, USA) was dissolved in 100 mL nonane to obtain a solution of $10^{-4}$ g/ml (0.1 g/L in nonane). The solution was further diluted in ethyl alcohol with the volume ratio of 1:10 and a perylene solution of $10^{-5}$ g/mL was obtained. Then the solution was finally diluted in distilled water with a volume ratio of 1:10000 to obtain testing samples with a concentration of $10^{-9}$ g/ml (1 µg/L). Then the sample was loaded in the cartridge and tested on the centrifugal SPE platform. The reagents and procedures were identical with the previous test with anthracene. Figure 3.14 shows the fluorescence spectra before and after the enrichment of perylene in water.

The diagram shows that perylene emits fluorescence with wavelengths between 420 nm and 580 nm. The peak of fluorescence intensity of SPE enriched sample was about 10 times of the original one before SPE. The integrated fluorescence intensity between 420 nm and 580 nm was calculated and compared with the original sample. The eluent after SPE with the prototype 3D cartridge was 8.30 times of the original sample. Similar with the previous test with anthracene, analyte (perylene) was completely extracted by the sorbent
in the microfluidic SPE cartridge, but not completely desorbed from the sorbent during the eluting process.

![Fluorescence spectrum before and after SPE](image)

**Figure 3.14.** Fluorescence spectra before and after the enrichment of perylene in water.

### 3.4. Conclusions

In centrifugal fluidic system, passive valves are commonly needed. However, most passive valves are normally closed and can only be sequentially opened by gradually increasing the rotational speed of the centrifugal platform. Siphon valves can work in the reverse way but their slender micro-channels can hardly be fabricated with current 3D printing technology. This chapter reported a novel gravity valve with three-dimensional structure, which helps to provide flexible use of different rotation speed in process control. Taking advantage of the 3D printing technology, gravity valve, Coriolis switching valve, and capillary valves are integrated in a single centrifugal SPE cartridge and 3D printed. The gravity valve allows the cartridge to rotate at a high speed to minimize the residual water in sorbent, and then release eluent slowly at a low rotating speed. The SPE centrifugal platform required no external valving system. The aqueous testing sample and reagents were simply controlled by the spinning speed/direction of the cartridge. The cartridge was 3D printed with a Fused-Deposition-Modeling (FDM) printer using PLA as structural material. Solid phase extraction and fluorescence detection of anthracene, one of the PAHs, was performed successfully with the presented cartridge on the centrifugal platform with different concentrations. Anthracene concentrations of the testing samples were 1 µg/L, 5 µg/L, and 10
µg/L, respectively. Volume ratio of the sample and eluent was 10:1. Experiment result shows that the integrated fluorescence intensities (390 nm to 460 nm) of different samples after SPE were 7 to 11 times of those for the original samples. Perylene, another PAHs, was also tested on the platform with a concentration of 1 µg/L. The integrated fluorescence intensity was 8.3 times of the original sample. The reported platform can be potentially used as an automated portable on-site enrichment and detection device for PAHs and other pollutant in environment water.
Chapter 4. Test of oil-seawater mixed samples with the centrifugal platform

4.1. The fluorescence spectrum of seawater and oil seawater mixed sample

Seawater samples were collected from Gulf of Mexico on Sep 3, 2019. Figure 4.1 shows the location of the sampling station. The longitude and latitude coordinates the sampling station were -91.396633° and 29.1922833°, respectively. The seawater were filtered with 0.47 µm filter paper to eliminate phytoplankton and other suspension impurities.

![Figure 4.1. Location of the seawater sampling station.](image)

The collected seawater sample were processed for fluorescence excitation emission matrices (EEMs) on the Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon, USA). Figure 4.2 shows the EEMs of the seawater sample. It can be found from the graph that the seawater sample could absorb UV light and emit fluorescence from UV to visible light. The fluorescence is mainly caused by Colored Dissolved Organic Matter (CDOM), which is a complex mixture of hundreds or thousands individual substances result from decaying organic matter [69, 70, 71]. On account of comparison, 20 µL crude oil (ONTA) were mixed with 40 mL distilled water, and another 20 µL crude oil (ONTA) were mixed with 40 mL collected seawater. As a result, 5 ppm oil-water mixture and 5 ppm oil-seawater mixture samples were obtained. Figure 4.3 shows the EEMs of the oil-water mixture. Figure 4.4 shows the EEMs of the oil-seawater mixture. As shown in the graphs, the crude oil and CDOM have overlapped absorption and emission ranges. To distinguish them, principle components analysis (PCA) method may be applied. However, it requires high resolution...
EEMs obtained with expensive equipment and time consuming computation. So it is not suitable for on-site monitoring.

Fluorescence detections were implemented for seawater, oil-water and oil-seawater samples under the UV excitation light of 365 nm (Thorlab M365f1). The fluorescence spectrum was measured and recorded with an OceanOptics Flam-X-XR1-ES Spectrometer. Two optical fibres (Thorlab M41L01, 600 µm, 0.48 NA) were used to introduce the excitation light and collect the fluorescence. The fluorescence spectra were measured via Oceanview (OceanOptics, USA) with integration time of 1000 ms. All other fluorescence spectrum measurements in this chapter were also completed using the same equipment and configuration for the purpose of comparison.

Figure 4.5 shows the comparison of fluorescence spectrum of the seawater, oil-water mixture, and oil-seawater mixture samples with 3 different oil concentrations (10 ppm, 5 ppm, and 2 ppm). The dashed curve shows the fluorescence intensity difference between the oil-seawater mixture and seawater. As shown in Figure 4.5, this curve is basically the same with the fluorescence spectrum of the oil-water mixture. Therefore, the fluorescence spectrum of oil-seawater mixture can be viewed as the linear superposition of the fluorescence spectrum of CDOM in seawater and the fluorescence spectrum of the crude oil. However, it is very difficult to tell the existence and concentration of oil in seawater directly from the fluorescence spectrum under the monochromatic excitation light, because the distribution and property of CDOM in seawater vary a lot with time and location.

4.2. SPE of the seawater sample

As discussed in previous section, it is difficult to distinguish the fluorescence of crude oil and CDOM in the seawater sample. A possible solution is to separate the crude oil with CDOM first, and then conduct the fluorescence detection. It has been discussed in previous chapters that the PAHs were the major fluorophores in crude oil, and they could be extracted and enriched in eluent via SPE method. As a result, PAHs and CDOM in testing sample will be separated if the CDOM won’t be enriched in the eluent during SPE process.
Figure 4.2. EEMs of the seawater sample.

Figure 4.3. EEMs of the oil-water mixture.
Preliminary experiment was conducted to verify the feasibility of this method. The 3D printed centrifugal microfluidic platform presented in chapter 3 was used to conduct SPE process with the seawater sample. Same as the experiment presented in section 3.3.2, 50 mg C18 powder (Thermo Scientific, USA) was used as the stationary sorbent. Methonal and nonane (1 mL each) were used as conditioning buffer and eluent, respectively. 10 mL seawater sample was used as the testing sample to find out if the CDOM could be extracted from the sample and concentrated to the eluent. The experiment was conducted with the same procedure presented in 3.3.2. The blue curve in Figure 4.6 shows the fluorescence spectrum (raw data) of the eluent after SPE with our platform. The orange curve is the spectrum of pure nonane under the same condition. The peak around 410 nm shows the Raman scattering of nonane. The green curve shows the difference with the spectrum of eluent after SPE and the Raman scattering of nonane. This curve may be the fluorescence spectrum of CDOM collected in the eluent, or some other unknown fluorophores concentrated by SPE, or the combination of them. In any case, the fluorescence intensity
Figure 4.5. Comparison of fluorescence spectrum of the seawater, oil-water mixture, and oil-seawater mixture with different oil concentrations: (a) 10 ppm; (b) 5 ppm; (c) 2 ppm.
is always lower than 250 in the range of 400 nm to 650 nm as shown in Figure 4.6. On the other hand, the fluorescence spectrum of the original sample has a peak of more than 2000 around 480 nm as shown in Figure 4.5. And the integrated fluorescence intensity of the eluent, which is $1.22 \times 10^5$, is one order smaller compared with the original sample, which is $1.06 \times 10^6$. It means that the CDOM in the seawater sample could hardly be collected in the eluent (nonane) after SPE with our design.

Because the PAHs will be collected and concentrated in the eluent while the CDOM won’t, these two groups of fluorophores can be separated with SPE method. So it’s possible to use the centrifugal SPE cartridge presented in chapter 3 to enrich and detect the spilled oil in seawater.

![Fluorescence spectrum of the eluent after SPE with seawater](image)

Figure 4.6. Fluorescence spectrum of the eluent after SPE with seawater.

4.3. Enrichment experiments of oil in seawater and discussion

More SPE experiment was conducted to further verify the feasibility of using the 3D printed cartridge for enrichment of crude oil in seawater. The fabrication and preparation of the centrifugal SPE cartridges used in the experiment were exactly the same with the one presented in section 3.2.4 and 3.3.2.

The oil-seawater mixed samples were prepared using Pennsylvania light crude oil (ONTA, Canada) and seawater. Three sets of samples were prepared and the oil concentration of
each sample were 10 ppm (part per million), 5 ppm, and 2 ppm respectively. In the experiments, the organic eluent (1 mL nonane), pretreating buffer (1 mL methanol), and testing sample (10 mL) were preloaded into chambers a, c, and d respectively using syringes. The experiment process was same as one presented in section 3.3.2. The programmed rotating frequency and the function of each step were shown as Figure 3.11.

Samples with different oil concentrations (2 ppm, 5 ppm, and 10 ppm) were tested, and the test was repeated 3 times for each concentration. Figure 4.7 shows the fluorescence spectra of the eluent after SPE for samples with different oil concentrations. Crude oil typically emits fluorescence with wavelengths between 400 nm and 650 nm under the excitation of 365 nm. The curve showing the fluorescence spectrum after SPE was averaged with 3 repeated test. The Raman scattering of the solvents (nonane) were deducted to make the fluorescence spectra more accurate. As shown in Figure 4.7, the fluorescence intensity of the eluent after SPE increases as the oil concentration of the original sample increases. However, the fluorescence spectra of the eluents after SPE just share a similar profile but not identical. The reason may be that the components of the crude oil are too complex. PAHs, the major fluorophores in crude oil, are a group of compounds with aromatic rings. There are dozens of different PAHs and their derivatives in crude oil and each of them has its own fluorescence spectrum. And they may have different recovery ratios in eluent under different experiment conditions, such as different sample concentrations.

Table 4.1. Comparison of fluorescence intensity and enhancement ratio.

<table>
<thead>
<tr>
<th>Original Sample</th>
<th>10 ppm</th>
<th>5 ppm</th>
<th>2 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integration time</td>
<td>1000 ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integration range</td>
<td>400 - 650 nm</td>
<td>400 - 650 nm</td>
<td>400 - 650 nm</td>
</tr>
<tr>
<td>Fluorescence intensity (sample)</td>
<td>490803</td>
<td>277427</td>
<td>171071</td>
</tr>
<tr>
<td>Fluorescence intensity (eluent)</td>
<td>3358097; 3995611; 2874678</td>
<td>1495780; 1314919; 1220703</td>
<td>787007; 989021; 927586</td>
</tr>
<tr>
<td>Enhancement ratio</td>
<td>6.84; 8.14; 5.86</td>
<td>5.39; 4.74; 4.40</td>
<td>4.60; 5.78; 5.42</td>
</tr>
<tr>
<td>Average enhancement ratio</td>
<td>6.95</td>
<td>4.84</td>
<td>5.27</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.15</td>
<td>0.50</td>
<td>0.61</td>
</tr>
<tr>
<td>Confidence interval (90%)</td>
<td>1.93</td>
<td>0.85</td>
<td>1.02</td>
</tr>
</tbody>
</table>
Figure 4.7. Fluorescence spectra of eluent after SPE with oil-seawater sample of different concentrations.

As discussed previously, the fluorescence spectrum of oil-seawater mixture can be viewed as the linear superposition of the fluorescence of CDOM in seawater and the fluorescence of the crude oil. Therefore, the fluorescence intensities of oil-water mixture sample with different oil concentrations (as shown in Figure 4.5) were integrated and compared with integrated fluorescence intensities of eluents after SPE (as shown in Figure 4.7). Table 4.1 shows the detailed information of the comparison. For the oil-seawater mixed sample of 10 ppm, the measured integrated fluorescence enhancement ratio of 3 repeated tests were 6.84, 8.14, and 5.86, respectively. As a result, the average enhancement ratio was 6.95 with a standard deviation of 1.15. The 90% confidence interval of the real mean enhancement ratio was $6.95 \pm 1.93$. For the oil-seawater mixed sample of 5 ppm, the measured integrated fluorescence enhancement ratio of 3 repeated tests were 5.39, 4.74, and 4.40, respectively. The average enhancement ratio was 4.84 with a standard deviation of 0.50. The 90% confidence interval of the real mean enhancement ratio was $4.84 \pm 0.85$. For the oil-seawater mixed sample of 2 ppm, the measured integrated fluorescence enhancement ratio of 3 repeated tests were 4.60, 5.78, and 5.42, respectively. The average enhancement ratio was 5.21 with a standard deviation of 0.50. The 90% confidence interval of the real mean enhancement ratio was $5.21 \pm 0.85$. The diagrams in Figure 4.7 show the fluorescence spectra of eluent after SPE with oil-seawater mixed samples of different concentrations. The spectra are color-coded for easier distinction: 10 ppm (blue), 5 ppm (red), and 2 ppm (green). Each spectrum represents the integrated fluorescence intensity across different wavelengths (nm).
ratio was 5.27 with standard deviation of 0.61. The 90% confidence interval of the real mean enhancement ratio was $5.27 \pm 1.02$.

Figure 4.8 shows the comparison of integrated fluorescence intensity from 400 nm to 650 nm and enhancement ratios of different oil concentrations. The red curve shows the average measured enhancement ratio of sample with different analyte concentrations. Error bar shows the 90% confidence interval of the real mean enhancement ratio.

![Comparison of integrated fluorescence intensities and enhancement ratios](image)

Figure 4.8. Comparison of integrated fluorescence intensities and enhancement ratios of samples with different oil concentrations.

As discussed in previous chapters, the oil in testing sample (or PAHs) was not completely recovered to the eluent. But the integrated fluorescence intensities of the eluent after SPE with presented centrifugal platform were still be enhanced to about 4.8 to 6.9 times of the original samples. And the SPE process not only enriched the oil in sample, but also help the researcher get rid of the interference of the CDOM in the seawater.

### 4.4. Conclusions

The seawater sample could absorb UV light and emit fluorescence from UV to visible light due to the existence of Colored Dissolved Organic Matter (CDOM). The crude oil and CDOM have overlapped absorption and emission ranges. So, it is very difficult to tell the existence and concentration of oil in seawater directly from the fluorescence spectrum under the monochromatic excitation light. The CDOM can hardly be collected in
the eluent after SPE. Thus, PAHs, the major fluorophores in crude oil, can be separated and enriched with SPE method and then be detected with fluorescence method. So it’s possible to use the centrifugal SPE cartridge presented in chapter 3 to enrich and detect potential spilled oil in seawater. Solid phase extraction and fluorescence detection of crude oil was performed successfully on the presented centrifugal platform with different oil concentrations. Oil concentrations of the oil-seawater mixed samples were 10 ppm, 5 ppm, and 2 ppm, respectively. The volumes of the oil-water mixed sample and eluent adopted in the experiment were 10 mL and 1 mL respectively. Experiment result shows that the integrated fluorescence intensities (400 nm to 650 nm) of different sample concentrations after SPE were 4.8 to 6.9 times of those for the oil-water mixed samples. This section further verified the availability and merit of the reported centrifugal platform in the field of on-site enrichment and detection of certain pollutant in environment water.
Chapter 5. Summer and future work

5.1. Summary

Solid phase extraction (SPE) is a widely used approach to enrich the analyte from aqueous sample. However, conventional SPE usually need to be conducted in laboratory with sophisticated instruments and well professional operators. Taking advantage of centrifugal microfluidic platform and 3D printing technology, the research work in this dissertation targets to develop a miniaturized automatic instrument based on SPE principle for enhanced detection of oil contents in water or other analytes. Several different types of valving techniques were applied on the centrifugal platform to help control liquids in the cartridge. Different analytes were tested and the presented designs showed good performances.

Firstly, a centrifugal microfluidic platform was built for spilled oil enrichment and detection. The sample and reagents in the cartridge were driven by centrifugal force and controlled by a mechanical valving system beneath the cartridge. The pinch-valves were simply controlled by the spinning frequency of the centrifugal platform. The prototype of the proposed system was 3D printed with an FDM 3D printer. The platform was tested with standard oil-water mixture sample of different concentration. In the experiment, C18, activated charcoal, and 3D printed porous polymer were used as stationary sorbent respectively and compared. The volumes of testing sample and eluent were 6mL and 0.6mL. The 3D printed porous polymer showed very promising performances with about 6 times of enrichment efficiency. The experiment results demonstrated functionality of the prototype lab-on-CD system.

To further improve the enrichment efficiency and reduce the weight and size of the centrifugal system, efforts were made to eliminate the mechanical valves and actuation system. The new design adopted a novel gravity valve which helps to simplify the overall design of the system. The centrifugal platform was designed to be a truly three-dimensional one. Compared with traditional capillary valves, which could only be opened by increasing the spinning frequency, the gravity valve was more flexible. Gravity valves, traditional capillary
valves, and Coriolis switching valves were integrated in a 3-dimensional microfluidic cartridge. All the valves were simply controlled by the spinning direction and frequency of the platform. The prototype microfluidic cartridge was fabricated in 3D printing technology, which helps to lower the cost. The prototype device was then experimentally tested. Anthracene and perylene, which were two of the Polycyclic Aromatic Hydrocarbons (PAHs), were used as the analyte and tested with the presented design, respectively. C18 was used as the stationary sorbent and fluorescence detection was applied. The volumes of testing sample and eluent were 10mL and 1mL. The original testing samples of anthracene were 1 µg/L (1 ppb), 5 µg/L, and 10 µg/L (anthracene/water), respectively. The original testing sample of perylene was 1 µg/L (1 ppb). The experimental results confirmed that the prototype device performed well and integrated fluorescence intensities after SPE were 7 to 11 times of the original sample for anthracene, and more than 8 times for perylene.

Coloured Dissolved Organic Matter (CDOM), which is a complex mixture resulted from decaying organic matter, can absorb UV light and emit fluorescence. The crude oil and CDOM in seawater sample have overlapped absorption and emission spectrum. Thus, it’s not easy to directly distinguish them and tell the existence and concentration of oil in seawater. However, the CDOM can hardly be collected in the eluent after SPE. Therefore, oil particles (or PAHs) can be separated and enriched with SPE method and then be detected by measuring fluorescence intensity. SPE process and fluorescence detection of crude oil was performed using the prototype 3D microfluidic cartridge fabricated using 3D printing technology. In our experiments, the oil-seawater mixed samples were prepared with the concentrations of 10 ppm, 5 ppm, and 2 ppm, respectively. The volumes of the oil-water mixed sample and eluent used in the experiment were 10 mL and 1 mL respectively. Experimental results showed that the integrated fluorescence intensities (400 nm to 650 nm) of eluent after SPE ranged from 4.8 to 6.9 times of the original oil-water mixed samples. The SPE process not only enriched the oil, but also eliminated the interference of the
CDOM. Therefore, the reported centrifugal platform can be potentially used for on-site enrichment and detection of certain pollutants in environment water.

5.2. Future work

The experimental results obtained so far with the prototype systems have demonstrated that the SPE efficiencies are still lower in comparison with those obtained using traditional SPE equipment. The experiments have proved that this is mainly caused by the low efficiency of elution. In the next stage of research, efforts will be made to modify the design of the centrifugal systems and to optimize experimental parameters to improve the eluting efficiency. For example, after the testing sample passes through, the residual water in the sorbent chamber tends to reduce the efficiency of the eluting process and also negatively affect the fluorescence spectrum of the eluent after SPE. Design modifications therefore need to be made to minimize the residual water.

Another potential factor that may negatively impact the performances of the system is the errors in fabrication of the microfluidic cartridge. For example, the cross-sectional area of a micro channel fabricated using the 3D printing technology tends to be smaller than the designed value. In addition, the cross-sectional profile of the micro channel is usually irregular though it is designed to be square or rectangle. When the designed size is smaller than $200 \mu m \times 200 \mu m$, the printed flow channel is usually blocked and resulted in a defective microfluidic cartridge. These can be improved by modify the parameter configuration of the 3D printing. Meanwhile, efforts need to be made to calibrate for the fabrication errors, and compensate for the errors. A better 3D printing machine with high resolution may also help to improve the fabrication precision.

In conclusion, this research has proved feasibility of using the centrifugal microfluidic system for SPE extraction and enrichment of oil or PAHs contents in water for fluorescence detection purpose. The long-term goal of the research is to integrate the fluorescence spectrum detection unit, which is currently under development in our group, into the centrifugal system.
Appendix A. Copyright Information

A 3D printed centrifugal microfluidic platform for spilled oil enrichment and detection based on solid phase extraction (SPE)

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3D printing fabrication and test of a centrifugal cartridge with an integrated gravity valve for solid phase extractions

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References


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Yong Zhang was born in Dezhou, Shandong Province, China. He received his bachelor’s degree and master’s degree from School of Electronics and Computer Technology at North University of China, in 2010 and 2013, respectively. He worked on MEMS since 2010. In 2014, he joined Dr. Wanjun Wang’s research group at Louisiana State University. His main research interests include microfluidic system, micro-optic system, mechanical system, etc.