MASTER OF BIOMEDICAL ENGINEERING

Feasibility study on enhancing the bio-sensors detective limitation of anionic surfactants

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Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

Signature: 

Date: 03/12/2018

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Chapter 1: Introduction **7**

Chapter 2: Literature review **10**

2.1 History and production 10
2.2 Healthy and Environment effect 13
2.3 Effect on the sexual and fertility function 14
2.4 Animal test results and in vitro results mechanisms 15
2.5 Standards of detecting PFOS/PFOA in drinking water and recreational water 18
2.6 Fluorescence 20
2.7 astkCARE 21
2.8 VFD Technology 23

Chapter 3. Methodology and results **28**

3.1 Materials 28
3.2 Fluorescence Method 32
3.2.1 Samples Preparation 32
3.2.2 Fluorescence test 34
3.3 The VFD technology 37
3.3.1Samples preparation 37
3.3.2 Rotation Speed effects 38
3.3.3 Residual time 40
3.3.4 Rest Time 41
3.3.5 Cycle Time 42
3.3.6 Best Conditions Determination and quantitative relationship building 43

Chapter 4: Discussions **45**

4.1 Fluorescence Method 45
4.2 VFD Method 46

Chapter 5. Feature Work **49**

Chapter 6. Conclusions **53**

Reference **54**

Figure and table list

Figure 1 The "Family tree" of PFASs [1] **10**
Figure 2 PFOS/ PFOA in human beings' body **13**
Figure 3 Typical symptoms of mice **16**

Figure 4 Symptoms in rat livers 16
Figure 5 A typical HPLC-MS system 19
Figure 6 astkCARE kit 21
Figure 7 VFD device 24
Figure 8 Agilent Cary Eclipse 9800A 28
Figure 9 Agilent Cary Eclipse 60 29
Figure 10 Hospital Pump 29
Figure 11 astkCARE kit 30
Figure 12 UHMWPE test tube 31
Figure 13 Fluorescence intensity of EV aqueous(32 mg/L) before and after adding excess PFOS 32
Figure 14 Fluorescence intensity at the different level of PFOS in 32 mg/L EV aq (0.1 to 2 ppm) 35
Figure 15 Fluorescence intensity at different level of PFOS in 32 mg/L EV aqueous(0.01 to 0.1 ppm) 36
Figure 16 Fluorescence intensity Vs Concentration of PFOS 37
Figure 17 Extraction efficiency VS rotation speed 39
Figure 18 Extraction efficiency VS Flow speed 40
Figure 19 Extraction efficiency Vs Rest time 42
Figure 20 Cycle time Vs Extraction efficiency 43
Figure 21 Nominal reading value after VFD Vs Real concentration 44
Figure 22 Nominal reading value Vs rest time 49
Figure 23 Hand-powered ultralow-cost paper centrifuge [42] 51
Table 1 Composition of preparing each concentration 33
Table 2 Composition of preparing each concentration 38

Abstract

The perfluoroalkyl substances (PFASs) are a kind of fluorinated organic chemicals which were firstly synthesised in the 1950s. Due to their unique chemical and physical properties, they have been chosen for several purposes, such as firefighting form and non-stick coating. Their worldwide commercial uptake has dramatically increased its usage in the past 70 years, drawing researcher's attention towards different aspects of PFASs such as their manufacturing method, analysis method, applications, but most importantly, the health effects of PFASs in water systems. The results of many studies show strong relationships between the level of PFASs and several serious illnesses including cancers of different types. Therefore, the US environment protection agency (EPA) had decreased the detected content level of PFASs in the drinking water several times to 0.07 ppb to prevent more illnesses from being developed. Other developed countries such as Germany and France have followed the US's action to reduce the level of PFASs in the drinking water, at the same time, it is believed that Australia will plan to revise the related acts.

Facing this urgent and indeed need, high-performance liquid chromatography (HPLC) is still the golden standard for detecting the PFASs in the water, but the disadvantage of using the HPLC is its requirement of a well-trained operator, time-consuming process, huge size and high cost limit its application from being implemented from onsite occasions. The astkCARE kit was a portable device which was invented to detect the concentration of PFOS/ PFOA in water by driving the patented reagent and capturing a picture through a smartphone's camera. The advantage of astkCARE is in its portability and acceptable accuracy, but the detection limit of astkCARE kit still does not extend to the requirement of US EPA, and thus there is potential to further optimize the size and structure of the astkCARE kit.

In this thesis, the existing bio-sensor device is optimised with (1) the VFD (vortex fluidic technologies) technology to increase the effectiveness of extraction process during the sample pre-treatment, (2) introducing the fluorescence detection method. With those optimisations, the preliminary results are very promising with enhanced anionic surfactants detection sensitivity. Further works will focus on the miniaturization of the device without additional power and the autonomous real-time measurement.

CHAPTER 1: INTRODUCTION

The Perfluorooctanesulfonic acid (PFOS) and Perfluorooctanoic acid (PFOA) are belonging to a group of man-made chemicals known as the per-and poly-fluoroalkyl substances (PFCs) [1]. PFCs had been invented and manufactured for a series of commercial and military usages including the household customer products like non-stick pans and fire-fighting foam, as the PFCs have very stable chemistry property and unique physical property. Due to these unique properties, the PFCs also have too long half-time and can be easily accumulated in human beings. Initially, scientists and the public did not pay much attention to these kinds of substances, as this group of man-made chemicals were expected to be harmless to the environment and human beings. With more studies and researches investigating the effect of PFOS/ PFOA over the last 30 years, a relationship was founded between the concentration of PFOS/ PFOA and diseases including cancers and metabolic diseases have been confirmed, raising the publics' attention on this issue. Since then, most governments of developed countries have established new standards for limiting of PFOS/ PFOA as well as the recommended detection method for PFCs. Although the standards and detection methods suggested could guide the public and related agencies to make the right decisions, considering the widely use of this group of chemicals in different situations and various places, and the strong evidence of its effect on the human being and environment, it is necessary to develop a portable, affordable and accurate detection method for PFCs for onsite use.

Before the experiments, previous related studies about the manufacturing history of PFCs are introduced, including the usage and effect of PFOA/ PFOS, and the history of detecting standards and development of detection methods in different countries and areas. To achieve our goal, a portable, affordable and accurate detection method. Some background of Vortex Fluidic Device (VFD) technology is also given as well as the fluorescent luminescence mechanism of a cationic dye and its combination with an anionic surfactant in the water solution or the organic phase. After comparing the current PFOS/ PFOA detection methods, comparisons between current methods for detecting PFOS/ PFOA and capturing the response to the absorption or intensity signal of PFOS/ PFOA itself are explored. This project aims to measure very low-level concentrations of PFOS/ PFOA, however the portability of the device is a contradicting

the requirement of detecting low-level concentrations. The size of key components of current devices are driving the sensor which is basically based on the voltage-dependent signal amplification, and the size of that is in the inverse ratio of the detection limitation and the sensibility. Therefore, this thesis aims to establish a method to find and establish which will achieve strong signal response when the concertation of PFOA/ PFOS is very low and the signal will decrease when the level of PFOS/ PFOA is increasing, i.e. the signal response is in inverse ratio of the concertation of the PFOS/ PFOA. The hypotheses made before the experiment is that there is a substance solution which has strong enough signal response to fluorescence or Uvi-vis absorption in a parable way, and the signal response will reduce when the PFOS/ PFOA was added in. So, a relationship can be established between the low concertation and the relatively strong signal response to decreasing the limitation of the detection method.

One of the issues in the current detection method is long pre-treatment time of the samples before it can be used for measurement. As it is known, in most situations where the samples collected were in the industry site, airport, firefighting training facilities. Samples from those areas are polluted by organic waste and metal ions as well. Before those samples can be used for the PFOS/ PFOA measurement, some relative influencing factors should be excluded to achieve a convincing result. The conventional method for pre-treatment is extraction by using a two-phase solution with reasonable efficiency. This method requires the sample to be shaken and rest for a long period to allow the solution to achieve a reasonable extraction efficiency. Although this requirement may be acceptable in the laboratory, the extraction efficiency and the ability might not be fully explored, which will be another factor affecting the final results. Meanwhile, the staff who is in a situation for on-site detection might not be able to follow the extraction introduction and wait for such a period of time to allow the solution to rest. To solve this problem, a device or technology is needed to simplify and accelerate the extraction process to achieve the extraction efficiency required.

To achieve a low detection limitation, the fluorescence method was driven to design the first experiments with relative strong signal response in the low concentration of samples. To shorten the pre-treatment time and enhance the extraction efficiency of

pre-treatment, the VFD technology was optimized to achieve the best conditions for the designed experiments.

In the experiment, sample solutions with a different level of PFOS/ PFOA have been prepared by strictly following the introduction given by the US EPA regarding the standard of detecting PFOS/ PFOA in the water system. For establishing the relationship between the concertation and the signal response, the variable factors are controlled to achieve the best conditions of the excitation wavelength, emission wavelength, slide time and PMT value. By following the best fluorescence conditions achieved, the intensity of different concertation was detected and recorded for analysis. For the method of shortening the pre-treatment time from VFD processing, the first step was to identify the affecting treatment parameter and find the best conditions for the treatment process. The new method and the previous portable method will be compared to evaluate the advantages of the new method.

CHAPTER 2: LITERATURE REVIEW

2.1 History and production

PFOS and PFOA are fluorinated organic chemicals which are parts of a larger group of substances referred to perfluoroalkyl substances (PFASs). The PFASs, which are also known as PFCs, are some synthesised substance developed in the 1950s by many companies around the world. The reason why the family of PFASs include a series of over 3000 manmade substances were synthesised in a brief time due to the chemical properties of fluorine with strong electronegativity and small size of the atom track size, leading the unique function and production capability of the PFASs. The "Family tree" of PFASs, including examples of individual PFASs and the number of peer-reviewed articles on them since 2002 are list in Figure 1[1].

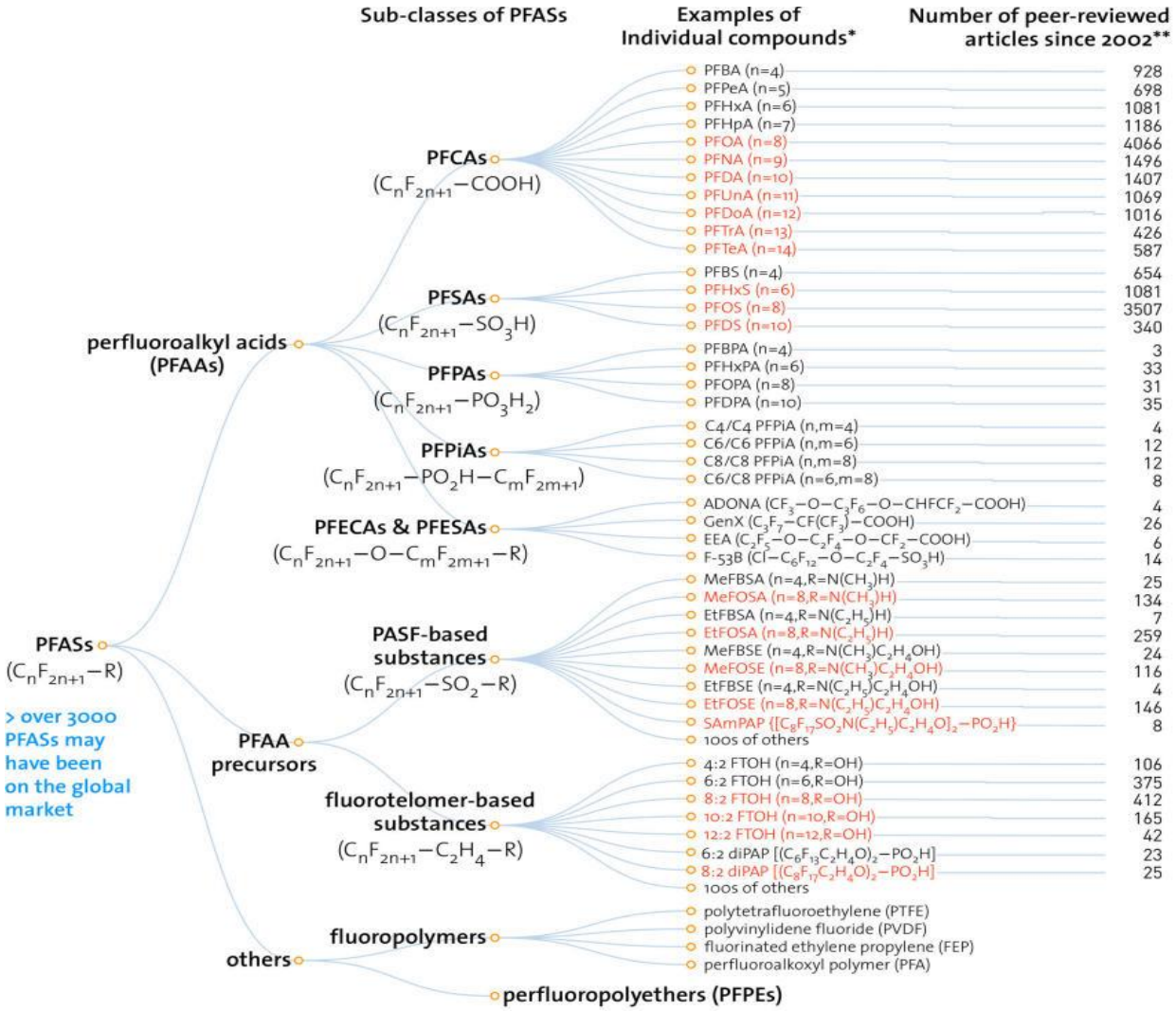

Figure 1 The "Family tree" of PFASs [1] from "A Never- Ending story of PFASs"

Since the properties of perfluoro carboxylic acids were discovered in the early 1920s, more researchers had made efforts into the synthesis of these kinds of substances. The properties were mainly affected by the extent and the method of combining the fluorine substitution, as the carbon-fluorine bond is the strongest bond and both the hydrophobic and oleophobic effect were given to the target substance [2]. Three chemistry structure-based properties aspects must be considered during the design of the chemistry substance: a) a high percentage of fluorine at the end of the target substance, such as perfluoroalkyl or perfluoropolyether [3-5]. b) a hydrophilic group such as hydroxyls. c) a link molecule to combine the two groups together [4]. For the industry synthesis of perfluoroalkyl and perfluoropolyether, the lead starting compound is usually hydrogen fluoride (HF) through electrochemical fluorination process or the synthesis from telomerisation of tetrafluoroethylene (TFE) [6]. The oligomerization of hexafluoropropylene oxide (HFPO) was chosen to synthesis the perfluoropolyether-based fluorinated surfactants as it is the recommended method [7-9].

The first research of synthesising the fluorocarbons was accomplished by J.H.Simons in 1949[10], which was driving the hydrogen fluoride and electric current in the employment of pyridine. The 1950s was the golden time for commercial production of fluorinated surfactants, including the PFOS/PFOA, which was drove by Simon's method (ECF) mentioned above. In this method, target substance sulfonated surfactants were typically divided into two parts for synthesis: a hydrocarbon sulfonyl fluoride and the corresponding perfluoroalkyl sulfonyl fluoride, such as perfluoroalkyl sulphonamide and perfluoroalkyl sulfonate, which could be synthesised by HF under the ECF method. There are several commercial perfluoroalkyl sulfonyl fluorides with carbon chain from 4 to 10 synthesised by these methods. The PFOS was hugely manufactured by this method in the middle of the last century. And one of the results is that: some parts of the carbon skeleton due to cleavage and rearrangement reaction was processed and the reason as to why the purity of this method is relatively low in comparison with the other methods during the following years. Another substance using the ECF process is PFOA. Usually, the starting compound is an alkyl carbonyl fluoride which is designed to yield to a corresponding perfluoroalkyl carbon fluoride which will react with the surfactant-like ester or amide.

There are several important and unique properties of fluorinated surfactant which cannot be alternative by other materials. The most well-known feature is that the

fluorinated surfactants could effectively decrease the surface tension when it was added into the aqueous solution in a very low percentage. Besides the general neutral solution, the basic, the acidic, and even some organic solvents were found to be effective when the fluorinated surfactants were added in. In term of the very low surface tension, fluorinated surfactants were used as fire-fighting foams or coatings which were designed to decrease the water solubility. The critical micelle concentration (CMC) is the concentration of surfactants above which micelles form and all additional surfactants were added to the system and is an important criterion to evaluate the functional ability of a surfactant [9]. The CMC of a fluorinated surfactant and the usual surfactants are almost the same, but as the length of fluorinated surfactant's carbon skeleton is shorter than hydrocarbon surfactant, the fluorinated surfactant could decrease the surface tension more efficiently, especially considering that it is of chemically inert of itself during reaction with other reagents. Another attracting property of fluorinated surfactants is that they show very low-level surface energy when the surface was given special treatment under an organic coating method on even very rough surface.

The unique properties mentioned above allowed the commercialisation of fluoro surfactants to expanded faster than expected. One of the most widely commercial uses of the surfactants is in the firefighting foams to put out polar and non-polar solvent fires [7]. As mentioned above, the principle of the fire-fighting foam is that the very low density will help them to deposit on the surface of the burning substance, which will result in the mass evaporation generated by the heat. This method is an efficient way to prevent the dangerous vapours from being separated. More than one fluorinated surfactants existed in the firefighting foam but also including the PFOS/PFOA which play an important role in forming the film above the solvent.

As the firefighting form was widely used in different military bases, the defence department had been noticed by the environment protection agency (EPA) that they should test the groundwater near the military base. The EPA could monitor the samples in their laboratories, but the defence department requires a portable device to detect the PFOS/ PFOA on site to determine if the groundwater had been polluted after their exercises or necessary actions.

2.2 Healthy and Environment effect

As the unique properties mentioned above, perfluoroalkyl compounds (PFCs) were widely industrially manufactured for many consumers' products including oil-resistant film and coating of the cooking tool and fire foam. These chemical properties are due to the strongest nature C- F bond existing in the PFCs, which leads to another side effect of themselves: they are non-degradable in the natural environment and will easily accumulate in the water cycled system and even in the blood circulation system of humans through the intake of food and water.

There were 23 kinds of PFCs that have been synthesised since the 1950s, most of which have more than 6 carbon chains. In the mainstream acknowledge, the PFOS and PFOS had attracted the public attention [11]. In previous researches, PFOS/ PFOA had been found in the most of human beings' body fluid which includes the serum, seminal fluid, breast milk, saliva, tears and even the umbilical cord, which is an evidence of PFOA/PFOS' s long half-life [12-15], shown in Figure 2.

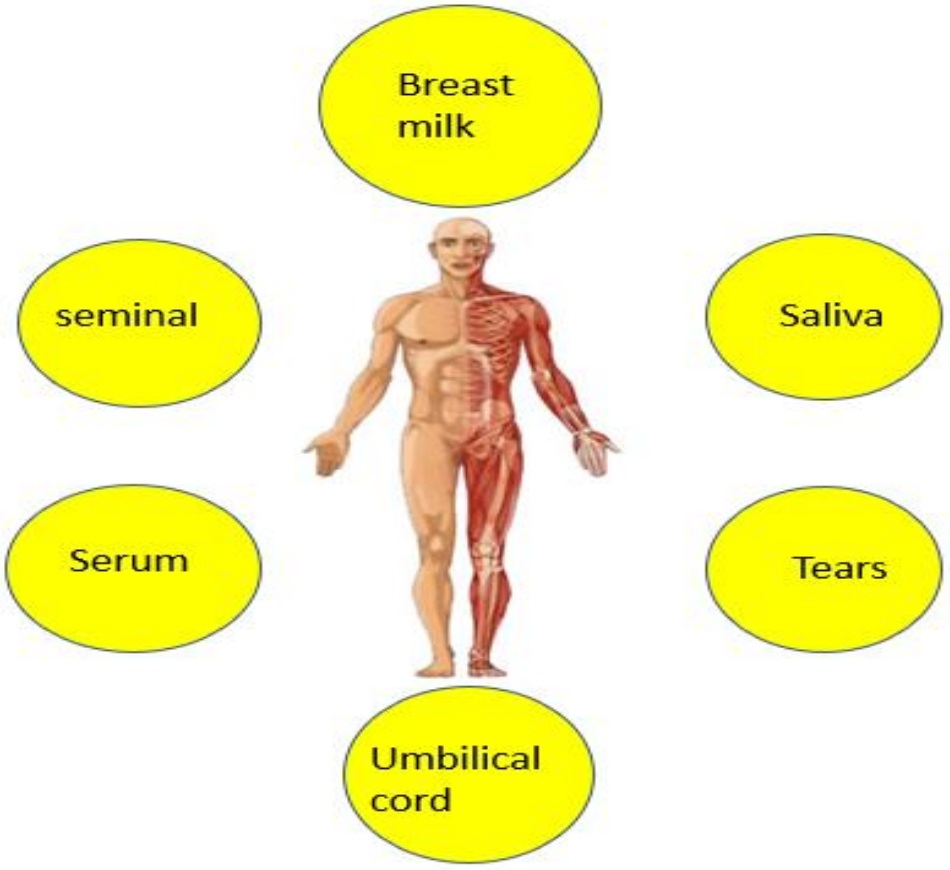

Figure 2 PFOS/ PFOA found existing in human beings' body

The PFOS/ PFOA has also been identified in the other parts of the human beings when the patients' health conditions faced some issues, such as respiratory disease, cardiovascular disease and immune system disease. In these diseases development, PFOS/ PFOA had been verified to play a vital role in being an inhibitor of the endocrine system or cell differentiation, especially for a newborn infants with the low function of the immune system or the elderly with degraded functions [16]. Other research had initially shown the mechanism of these to be harmless, basically because of the PFOS/ PFOA hormone-like substance act on the receptor which was originally combined with the central hormone regulation cell.

2.3 Effect on the sexual and fertility function

Previous studies on the relationship between the exposed individuals under PFOS/ PFOA and the human fertility functions mainly focused on the vitro and animal study which had given a significant evidence regarding side effects. For adult men, the results showed that PFOS/ PFOA will reduce the function of testicular by preventing the frequency of steroid synthesis in vivo [17-21]. According to their studies, the quality of attended adult men's semen showed that there were morphological differences and the number of sperm decreased under the presence of PFOS/ PFOA appearing combined or individually. Further chromosome research showed that the effects of PFOA/ PFOS started by changing the mitosis stage of the cell [22-24]. Despite the effect on the cell itself, PFOA/ PFOS display its toxicity as an inhibitor on the central nervous-hypothalamus system which could reduce the activity of hormone secretion. Without the hormone normal secretion, sperm concentration of middle age men would decrease and as a result, the sexual and fertility ability of males will be lower than those without PFOS/ PFOA exposure. The gene expression of the hormones was also induced by the presence of PFOS/ PFAS in the blood system, especially for the end part of the hypothalamic cell, the higher level of PFOS/ PFOA will inhibit the activity of a receptor of the cell from testis. The reasons why the PFOS/ PFOA could go through to the blood-testis barrier is still not clarified and the possible mechanism is the blood-testis barrier function being reduced by the higher levels of PFOS/ PFOA damaging the cell connection between them [25-26]. In a previous animal control test, infertile male mice showed that PFOS/PFOA levels in of blood were higher than the controlled mice.

This fact is another evidence of PFOS/ PFOA's side effect on sexual and fertility function.

2.4 Animal test results and in vitro results mechanisms

Except for the direct evidence of damage for human beings, there are numerous previous experiments showing positive results between animal tests or in vitro results, and some possible mechanisms were given. A study [27] which was designed to give mice specific level PFOA with peanut oil showed pancreas and liver damage of different degrees corresponding with the concentration of PFOA when compared against the control team. The damages mainly appeared in these following aspects: 1) although the weight of mice did not show a significant differences, the liver tissue in the test group showed swelling in different tends which indicated that the damage is related with PFOS in different concentrations. 2) Further biochemical analysis showed the liver tissue of test group mice contains a higher level total amount of enzyme (gamma-glutamyl-transpeptidase), which is a key feature of liver damage. 3) Blood test for glucose levels after fasting the mice showed that the metabolic dysfunction of the liver was reduced in the PFOS-tested group, which is the main feature found in most types of liver cancer. The possible mechanisms of the PFOS effect on liver tissues were given by cell morphology under a microscope which showed the number of specific liver cell and the morphological changes in the living mice liver tissue. The results showed that the liver cells of the PFOS-tested group had twice the amount of CD-36 cell. By conducting the immunoassay on the damaged liver cell, the results showed that the FGF21-immunolabeled liver cells despair and the western blot result showed that decreasing regulation happened in the liver cell of PFOS-tested mice. By attaining these results, the conclusion was made that the exposure of excess does of PFOS would damage the healthy liver cell by reducing the cell growth and affect the DNA self-repairing process which is the main reason for the development metabolic-related tumour.

Another quantitative research [28] focused on the accumulation differences between PFOS and PFOA on target organs of mice such as kidney, liver, lung and circulatory

system, and the damage degree of these organs. Generally, some abnormal activities appeared which include a reduction in activity, less food and water, and the weight of mice shows growth compared against that of the controlled group. Specifically, for the liver, the dark red colours were found on the surface of mice in the 20mg/kg/day dose group, which are shown in Figure 3.

Figure 3 Typical symptoms of mice after given 20mg/ kg/ day PFOS[28]

For the weight increase of the liver was found to be proportional to the additional dose of PFOS, although the toxicity mechanism of PFOS is not totally verified, it is widely accepted that the enzymes' activity of liver was reduced and the protein within the liver was affected by absent of the self-repairing ability of DNA. Most lung damages were due to high accumulation of PFOS which were concentrated on the surface of the lung, and lead to overload breathing and unconscious internal bleeding around the face and mouth of mice. All these symptoms are shown in Figure 4.

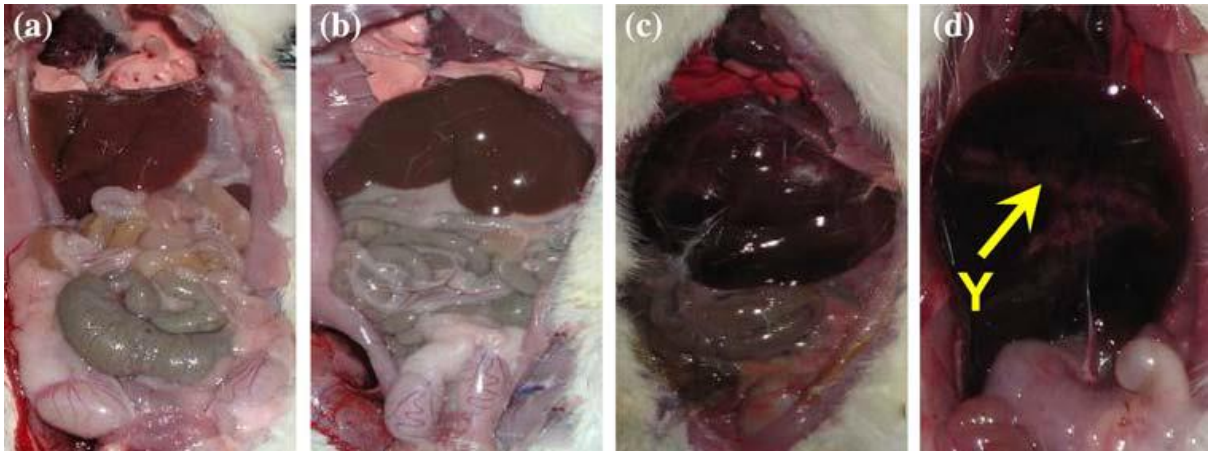

Figure 4 Symptoms after increasing dosage PFOS in rat livers[28]

A possible reason for this was hypothesized such that there were not enough alveolar cells being produced, which is an essential substance to maintain the lung function, as the top surface of the alveolar cell were covered by PFOS. Increased concertation of PFOS appeared around the kidney was indicating that the kidney was also affected. Comparing the high dose PFOS-tested group against the control group, an unconscious silhouette and enlargement were found in the epithelial tissue of near end kidneys in the high dosage group. These phenomena could indicate that the exposed mice may have endocrine disruption as the possible reason could be that cytoplasmic acidophilus was enhanced by being activated through the pathway of the renal cortex. For other target organs which include the blood system, testicle and brain, the accumulation of the PFOS will affect those to tissue in different degrees corresponding with the concertation of PFOS. By analysing the concentration of different organs, the liver and kidney were the primary targets which meant that the liver and kidney cell is more sensitivity to PFOS. The accumulation in the brain increased tremendously when the concentration was higher, which could be explained by the higher level PFOS meant that more successfully went through the blood-brain barrier. The transporter for PFOS from renal to urine is activated by the sexual hormones which are enhanced by PFOS in the blood system and wiped out by urinary generation process.

2.5 Standards of detecting PFOS/PFOA in drinking water and recreational water

As more evidence has confirmed that, PFOS/ PFOA has significant and effect on the human being and environment, most governments of developed countries had established the related standards and detection methods of PFOS/PFOA in the drinking water or recreational water. These standards or methods were established based on the different situations including the chance of exposure, and the public water purification system from of the various counties or areas.

For the United States, the EPA first gave the public a provisional health advisory regarding PFOA/ PFOS which first indicated the daily maximum intake amount of PFOS being 0.2 μg/L taken from the toxic dynamic experiments between animals and human being in 2009. In 2014, the US EPA then prepared a document on the health effects of PFOA/ PFOS. In that document, the first comprehensive toxicity assessment of the effect on human beings was given by summarising of the results of previous researches being conducted. The newest document given by the US EPA is a technical fact sheet of PFOS/ PFOA. In this fact sheet, the results of previous toxicological studies on animals and epidemiological studies were given, and considering the amount of existing PFOS and PFOA in the environment and water system, the US EPA gave several standards doses of the PFOS/ PFOA. For the oral non-cancer reference doses, the recommended dose is 0.00002 mg/kg/day for both PFOS/ PFOA, furthermore, the established drinking water standard advisory is 70 parts per trillion (0.07 ppb). Meanwhile, the US EPA also gave the standard detection method of PFOS/ PFOA, which were mainly driven by the high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS), as shown in Figure 5, from the EPA method 537, Version 1.1 [29].

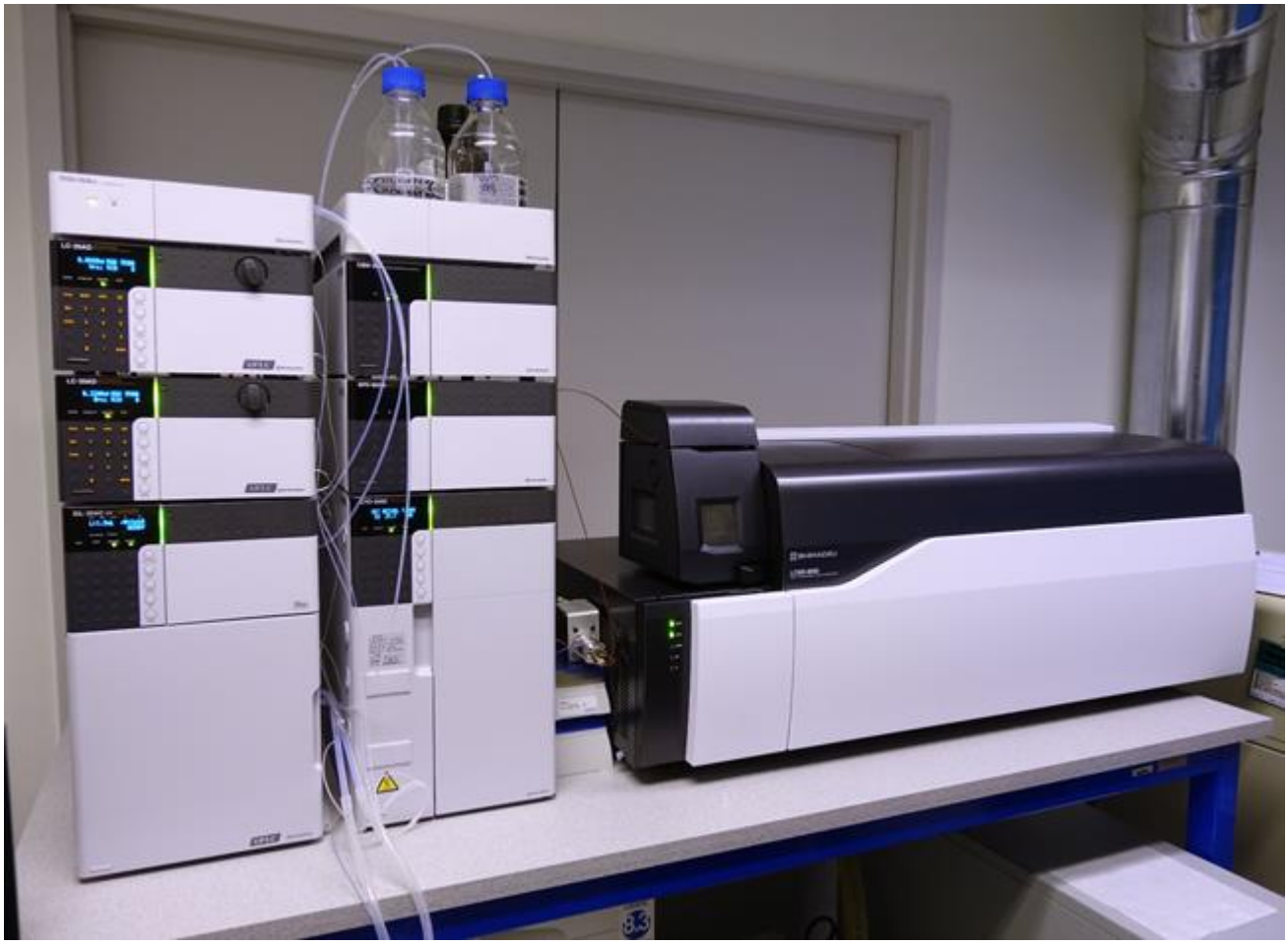

Figure 5 A Agilent Model 1200 HPLC system quad pump system with diode array and fluorescence detectors, auto-sampler and fraction collector.

The advantage of the HPLC-MS method is highly accurate as a standard. But the obvious disadvantage is that this method requires professional laboratory staff and the size of equipment is too large to move out for portable use, furthermore, the expanse of per sample is much more expensive (100-200 $). Another issue which needs to mention is that, unlike the other organic compounds, the PFOS/ PFOA is easy to adhere to glass, therefore the container should be replaced by the plastic container which is made of like polyethylene [30]. Also, the standard operating procedures including the pre-treatment of solid phase extraction. In addition to this, the detection method for another medium like soil, air and earth is given by using the LC-MS/ MS [31]. In South Australia, the information sheet about PFOS/ PFOA was given by the SA government EPA in 2017 after the health guidance was launched by the Australian government department of health in 2016. In this information sheet, the tolerable daily

intake does for PFOS/ PFOA are 20 ng and 160 ng respectively. For the drinking and recreational water, the sheet follows the standards of that given by US EPA.

2.6 Fluorescence

The first fluorescence substance was found in 1560 by Benmardino for his study on the substance derived from the wood of two tree species, which was later confirmed that the substance he found was matlaline [32-33]. At that time, the mechanism of fluorescence was not clear until the essence of light was explained in 1852 and the general concept of fluorescence with emission and excitation was widely accepted after the Planck's constant was confirmed [34-35]. Unlike the phosphorescent materials, the fluorescence substance will not emit when the radiation stops, which limited its early application. Currently, with the development of the lighting source and the detection method, fluorescence has more applications including in the areas of mineralogy, medicine, and biomedical sensors. The principle of fluorescence is that a special wavelength emission of light exist for each substance, which absorbs light at a some particular wavelength. As the energy of light is expressed by the Planck – Einstein relation Equation (1):

$$E = hf \left(f = \frac{c}{\lambda}\right), \quad (1)$$

Which meant that energy is only dependent on the frequency (wavelength). In general, the wavelength of emitted fluorescence of light is longer than the excitation wavelength, because there must be some energy loss during the time a photon is absorbed and when it one is emitted.

From the mechanism of fluorescence above, the advantage of the fluorescence spectrometer is obvious when compared to the other spectrometer such as Ultraviolet and visible light (UV/ Vis):

- Sensitivity: the sensitivity of fluorescence spectrometer is 1000 times greater than another absorption spectrophotometer for the special wavelength of emission and excitation wavelength, which generate a greater ability of detection limit.
- Specificity: the fluorescence method could only detect the substances with fluorescence, which means a better specificity than the UV/ Vis method.

- Detecting range: the detecting range of concentration can be diluted three or six logarithmic order times than the UV/ Vis method.

In general, the fluorescence spectrometer is expected to achieve a lower detection limit with high accuracy, to measure the level of PFOS/ PFOA in solution.

2.7 astkCARE

The astkCARE [36] is a sensitive and portable test kit based on the smartphone camera which was developed by the organization CRC CARE to detect the anionic surfactant such as PFOS/PFOA in the water system. The astkCARE kit is composed of three parts: the holding device, the patented reagent and the application in the smartphone as shown in Figure 6.

Figure 6 astkCARE kit work with a samsung smartphone

The holding device is a cylinder with space within that is made of aluminium and some polyethylene for the sample container. On the outside of the cylinder, the smartphone

holding part was attached with a movable bolt which allows it to be adjusted for the different smartphone model to ensure that the camera will be parallel to the right hole through the cylinder to capture the signal. On another side of the bottom, there are two white led lighters controlled by a switch outside the cylinder and powered by the battery underneath the bottom cover. When the cap of the holder is covered, these two led lights will provide pure background for the sample. The sample container is custom made by polyethylene with a cap on it, which permit the light to go through the container to capture the signal. The inner side of the cylinder was covered by the white paper to a minimum environment effect.

The patented astkCARE reagent is a mixture of acetone, ethyl acetate and ethyl violet (EV) aqueous in a fixed ratio, which is protected by the world patent WO2018039706A1. The EV is used as a cationic dye to combine with the AS (PFOS/ PFOA), and the organic phase is aimed to extract the combination from the water phase. Following the introduction of the patented reagent, the sample water and the patented astkCARE reagent are mixed together in a fixed ratio in the custom-made sample container and shaken before resting for detection. It is especially important to note that, the patented astkCARE reagent should be kept in the fridge and out of light.

The application of smartphone current support android operation system only, and the main function is to collect the signal and analysis the RBG (red, green and blue) light by targeting the red square area in the sample container which can be removed by the operator. When the measurement was taken, the signal of inner circulated RBG was achieved and saved. The signal collected is from the camera for the total amount of reflection spectrum. By driving different approaches, the repeated capture of the pictures is decoded to confirm the data of stable RBG. During the capture process, all the functions of the camera are shut down to decrease the other effect of the camera.

When the sample is ready, the patented astkCARE reagent and the sample were mixed before being transferred into the custom-made container to be shaken for a while, and then rest for 5 min to reach the established equilibrium between the two phases. The top layer of the organic phase contains the AS- dye combination, so the container was placed in the holder to be shot by the camera at the top of the upper organic layer. The LED lights were turned on, and the holding cap was covered to launch the application: the calibration function was chosen, ensure that the red square

sign at the top of the organic layer is aligned; the record function was then chosen to take a shot, and the signal will be recorded and analysed which include the GPS data allowing location of the samples to be identified.

The advantages of the astkCARE kit for detecting the PFOS/ PFOA is that this device is small in size to carry out for on-site measurement without any professional staff and the cost is very low when compared to the HPLC-MS method. On the other hand, the pre-treatment time is relatively long for a portable device used outside the laboratory, and there is more potential to enhance the accuracy of detection. To shorten the pre-treatment time of the extraction process, based on the previous research results on the two-phase extraction of C-phycocyanin from Spirulina maxima [43], the VFD technology will be driven as opposed to the shaking process.

2.8 VFD Technology

The vortex fluidic device (VFD) was invented by the Professor Colin Raston of Flinders University. Until now, this technology had been used in many fields including drug delivery system design, biodiesel manufacture, and other continuous synthetic processing. The dynamic thin films are one of the most important characteristics of the VFD which is generated by the continuous liquid being added into the container under the high-speed rotating conditions. The VFD device is shown in Figure 7.

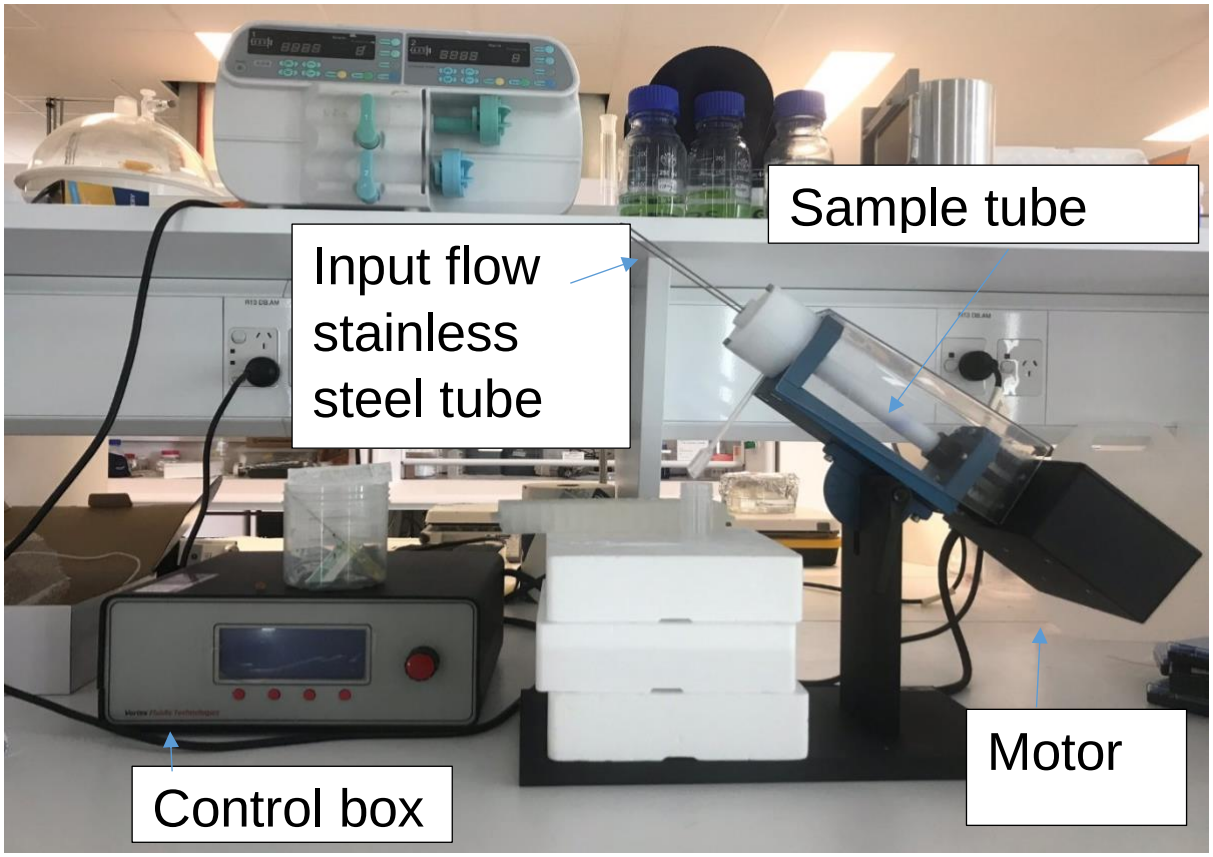

Figure 7 VFD device consist of motor, control box, sample tube

When the samples are slowly injected into the container, the centrifugal force given by the inner wall and the high-speed rotation will ensure that the liquid sample form a thin surface on the inner wall, and the friction given by container will extend the size of the liquid samples to make it thinner while the speed is increasing. In this environment, the substances mixed will be forced to a high level of macromixing and shear stress [37]. When these fluids were exposed to the sheer stress of the Stewarton/ Ekman layer, native vibration occurs. These environments provided by the unique physical properties will give the substance a better supporter for the chemical or physical reaction. Initially, researchers in this area expected that the spinning disk reactor (SDR) [38] will give the same effects of dynamic thin films. At that stage, the SDR and reverse tranche position RTP achieved similar results which appeared as expected, but the disadvantages of those are that it cannot provide a larger value processing ability. The purpose of inventing VFD is to establish a similar device with existing advantage and adding continuous flow capability. Comparing the VFD and SDR or RTP, it can be found that several improvements had been applied in the experiments

as a research tool. The first obvious advantage is that the VFD provides longer processing time for the continuous flow compared with that the previous two devices. Secondly, the VFD provides a wide range of liquid sample value from a drop to 50ml. Another important factor is the vibration effect, which appears when the high-speed rotation applied, as it will present some unexpected effects on the substances mixed with-in the container. The vibration phenomenon to the substance is reported several times before but still not applied in the experiment due to as it is hard to control for a specific environment. As the speed of the rotation is very high during the process, even a very tiny unexpected mismatch, such as the dislocation of the centre, will lead to the vibration of the whole system. Under the limited manufacture precision, this kind of vibration will increase while the rotating speed is raised. As the vibration increases, the liquid sample will provide a response as the molecular tension and intermolecular force which is determined by the property of the reagent itself. The response will result in a mechanical wave being generated, which is the main energy transportation to the substance in the reagent. Furthermore, there is another liquid dynamical reaction in the inner wall of the container which forms a microwave at the surface of the reagent. These two kinds of energy transformation will hold the sample surrounding the vibration of mechanical waves at a special condition for a particular rotating speed. The above environment established will give benefit to several processes including chemical synthetic, extraction and bio-transformation.

As the VFD could provide a unique environment as mentioned above, some types of organic transformations could be accelerated. One of the cases is the Diels-Alder reaction which is a basic organic reaction and usually used for total syntheses, with the most important fact being the speed and the conversion rate of the reaction [39]. The conventional method for Diels-Alder reaction is heating using an oil bath or sand bath, which will provide a continuous heating source to accelerate to the reaction. During the study of VFD-conditional Diels-Adler reaction, two important factors were considered, that is the tube’s inclination and rotation speed. The angle of the sample tube will affect the vibrations given by the inner wall of the tube. Specifically, when the samples’ tube was installed horizontally, the vibrations provided will be at maximum under the weight of this amount of sample could give; correspondingly, when the sample tube was installed vertically, the vibration from the inner wall of the tube will grow with an increase in the speed of rotation. After several experiments of controlled

variable methods, the angle of 45 degrees gave enough vibration to ensure that chemistry process happens faster. Another factor that should be considered is the continuous flow process of the sample being injected. Increasing the angle makes it harder to ensure the continuous flow of the sample. To maintain a continuous flow under a range of rotation speed from 4000 to 7000 rpm, the angle will be set at 45 degrees. For the rotational speed factor, in its expected range (2000 to 3000 rpm), the higher speed would result in a bigger shear stress being developed, which will lead to the yield of the reaction increase as expected, but over 4000 rpm, the increased shear force will form an unexpected effect to reduce the reaction's yield rate. In the following research [40], the rotational speed shows more important support for reaction processing yield at a specific speed. Under all those criterions, the yield of standard Diels-Alder reactions will be up to 75% from typically 15%. The biomedical transformations are another application which is also significantly increased by VFD.

These biomedical transformations include drug delivery, material folding and bio-reaction across the carrier, such as a cGPM dominated protein transportation. As the mechanical wave is the main carrier of the vibrations, the biomedical transformation process is determined by the speed of the rotation. A key factor of the biomedical transformation is the structure of enzymes which will be folded or deconstructed. For example, when the VFD generated vibration transform the mechanical wave of esterase, alkaline phosphatase, b-glucosidase and deoxyribose-phosphate aldolase (DERA) [40], the activity of the unfolded enzymes will be enhanced by high-level folding or structuring to accelerate the catalytic efficiency of the substrates. As the same criterions mentioned above, the specific rotation speed is required to maximize the catalytic efficiency which is determined by the different microstructure and corresponding properties. Another issue about biomedical transformation is the temperature of the action which is sensitive to temperature change. As we know, in the VFD process, a side effect of vibration is heat being generated form high-speed rotation. For the fluid flow process, this will not be a problem as the fixed flow speed of flow will ensure that the heat is transferred away from the tube, but if a small amount of enzyme was injected and kept in the inner walls of the tube, it is necessary to keep the environment at a stable temperature for the ideal reaction conditions.

In conclusion, the VFD technology has been successfully applied in many fields which were supported by many pieces of evidence shown. The acceleration of reactions was

verified by previous experiments related to the rotation speed and tube angle. The further application will be extended in more filed by providing temperature and pressure control along with further exploration into thin film’s reaction mechanism.

CHAPTER 3. METHODOLOGY AND RESULTS

3.1 Materials

All chemicals including the PFOS/ PFOA, ethyl violet and the organic solvents used such as acetone, methanol, ethanol, and ethyl acetate were purchased from Sigma-Aldrich Australia. Quartz cuvettes were purchased from Airekacells. Fluorescence spectrometer used was Agilent Cary Eclipse 9800A model, as shown in Figure 8.

Figure 8 Agilent Cary Eclipse 9800A

The UV-vis spectrometer used was Agilent Cary Eclipse 60 which is from the chemistry laboratory of Flinders University, shown in Figure 9.

Figure 9 Agilent Cary Eclipse 60

The springe pump used is a hospital use model with dule pathways, which is shown in Figure 10.

Figure 10 Hospital Pump

The astkCARE kit and astkCARE reagent are donated by CARA as shown in Figure 11.

Figure 11 astkCARE kit

Others related materials were purchased from the chemistry store at Flinders University.

As the tube and cap of the VFD were made from Teflon and glass, materials that will release and absorb the PFOS/ PFOA, the Teflon cover and glass tube had to be replaced by ultra-high molecular weight polyethylene (UHMWPE), which were manufactured by the Services Group Engineering (SGE) of Flinders University. The diameter of the tube was designed to be 1mm less at the contact part when compared to the original size, as the space between the materials of UHMWPE should be bigger than that between the UHMWPE and glass for dissipating the heat generated from the friction of high-speed rotation, which is shown in Figure 12.

Figure 12 UHMWPE test tube

As the strength of UHMWPE is weaker than that of the glass, the tube wall should be designed to be much thicker than the glass tube and considering the inner diameter for input and output flow, the inner diameter was designed to be 1.5mm, which is less than the original diameter of the glass tube.

3.2 Fluorescence Method

To detect low concentrations of PFOS/ PFOA in water, a method was tested by using a solution that could give out a fluorescence signal response. When PFOS/ PFOA is added, the fluorescence intensity should decrease. At a specific concentration region of PFOS/ PFOA, the relationship between the fluorescence intensity and the concentration will be established as a master calibration is used to detect the unknown sample's concentration.

Several cationic dyes solutions have been prepared and ethyl violet (EV) aqueous solution (32 mg/ L) had a fluorescence response with an emission wavelength of 408 nm when the excitation wavelength was set at 312 nm. Fortunately, the fluorescence signal disappears when the excess dose of PFOS/ PFOA has been added in the sample solution, as is shown in Figure 13.

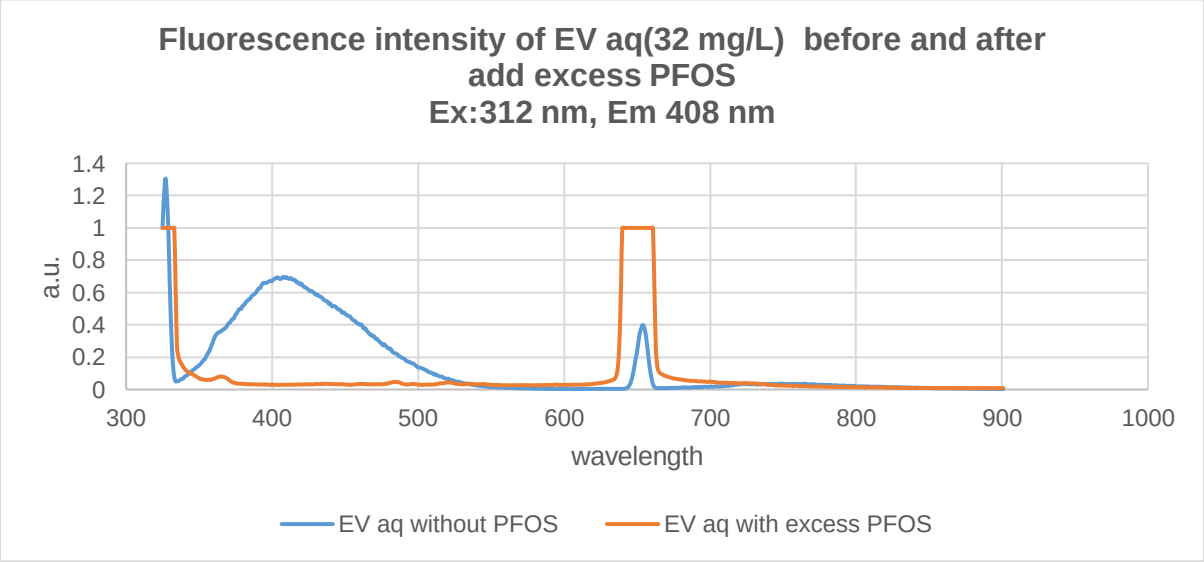

Figure 13 Fluorescence intensity of EV aqueous(32 mg/L) before and after adding excess PFOS

Based on initial investigation, the following experiment had been designed to observe the relationship between PFOS concentration and fluorescence intensity.

3.2.1 Samples Preparation

Firstly, 5 L of a mother solution had been prepared which of ethyl violet aqueous solution (32 mg/ L). 32 mg of ethyl violet was weighted before being, transferred into

a 1 L volumetric flask, and 1 L of milli-Q water (>18 MΩ) was added before being placed, into an ultrasound to fully dissolve for 10 mins, and left to rest at room temperature. The whole process was processed five times, with the solution being stored in a 5 L light protection reagent bottle covered with aluminium foil for following experiments. For the PFOA/ PFOA samples solutions, three concentration levels of 176 ppm, 1.76 ppm and 17.6 ppb have been prepared. All samples prepared above are placed in room temperature with light protection aluminium foil. The concentrations prepared for the PFOS/ PFOA are 10 ppm, 2 ppm, 1 ppm, 0.9 ppm, 0.8 ppm, 0.7 ppm, 0.6 ppm, 0.5 ppm, 0.1 ppm, 0.09 ppm, 0.08 ppm, 0.07 ppm, 0.06 ppm, 0.05 ppm and 0.01 ppm. The compositions used to preparing each concentration is listed in Table 1 below.

Table 1 Composition of preparing each concentration

	Ethyl violet (32 ml/ L)	PFOS/ PFOA	volumetric flask
10 ppm	95.32 ml	5.68 ml *176 mg/L	100 ml
2 ppm	98.83 ml	1.17 ml *176 mg/L	100 ml
1 ppm	47.16 ml	2.84 ml *176 mg/L	50 ml
0.9 ppm	47.44 ml	2.56 ml *176 mg/L	50 ml
0.8 ppm	47.73 ml	2.27 ml *176 mg/L	50 ml
0.7 ppm	48.01 ml	1.99 ml *176 mg/L	50 ml
0.6 ppm	48.30 ml	1.70 ml *176 mg/L	50 ml
0.5 ppm	48.58 ml	1.42 ml *176 mg/L	50 ml
0.1 ppm	84.1 ml	15.91 ml *1.76 mg/L	100 ml
0.09 ppm	85.68 ml	14.32 ml *1.76 mg/L	100 ml
0.08 ppm	87.28 ml	12.72 ml *1.76 mg/L	100 ml
0.07 ppm	88.86 ml	11.14 ml *1.76 mg/L	100 ml
0.06 ppm	90.454 ml	9.546 ml *1.76 mg/L	100 ml
0.05 ppm	92.045 ml	7.955 ml *1.76 mg/L	100 ml
0.01 ppm	48.409 ml	1.591 ml *1.76 mg/L	50 ml

Each sample was transferred into the corresponding volumetric flask, placed it into ultrasound to eliminate the bubbles for 5 mins, and left to rest at room temperature.

3.2.2 Fluorescence test

For the fluorescence spectrophotometer , there are three key conditions (the excitation slit, the emission slit, and the PMT voltage) that were required to be set up in order to achieve an expected graph that could get a medium intensity in the coordinate system and with a fluorescence peak that is easy to be identified. After several experiments were conducted on the samples prepared, three conditions for all samples had been chosen to be optimised:

1) Excitation slit: 5 nm; Emission slit: 5 nm; PMT voltage: 1000 v
2) Excitation slit: 10 nm; Emission slit: 10 nm; PMT voltage: 800 v
3) Excitation slit: 10 nm; Emission slit: 10nm; PMT voltage: 900 v

After measurements, the best results came from three series of data with conditions 2). With the excitation wavelength 312nm as the standard reference peak, the normalised fluorescence peak graph was generated as shown in Figure 14 and 15 below.

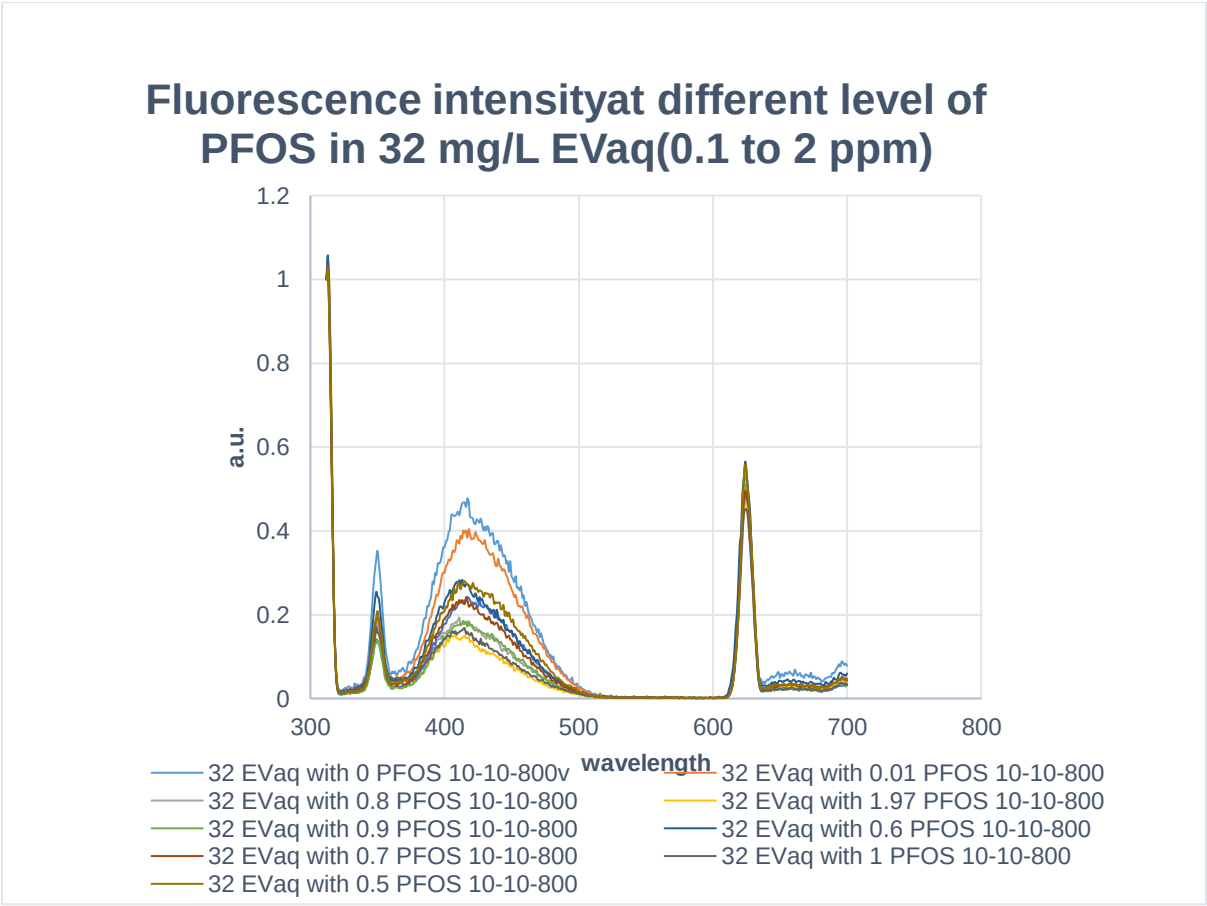

Figure 14 Fluorescence intensity at the different level of PFOS in 32 mg/L EV aqueous (0.1 to 2 ppm)

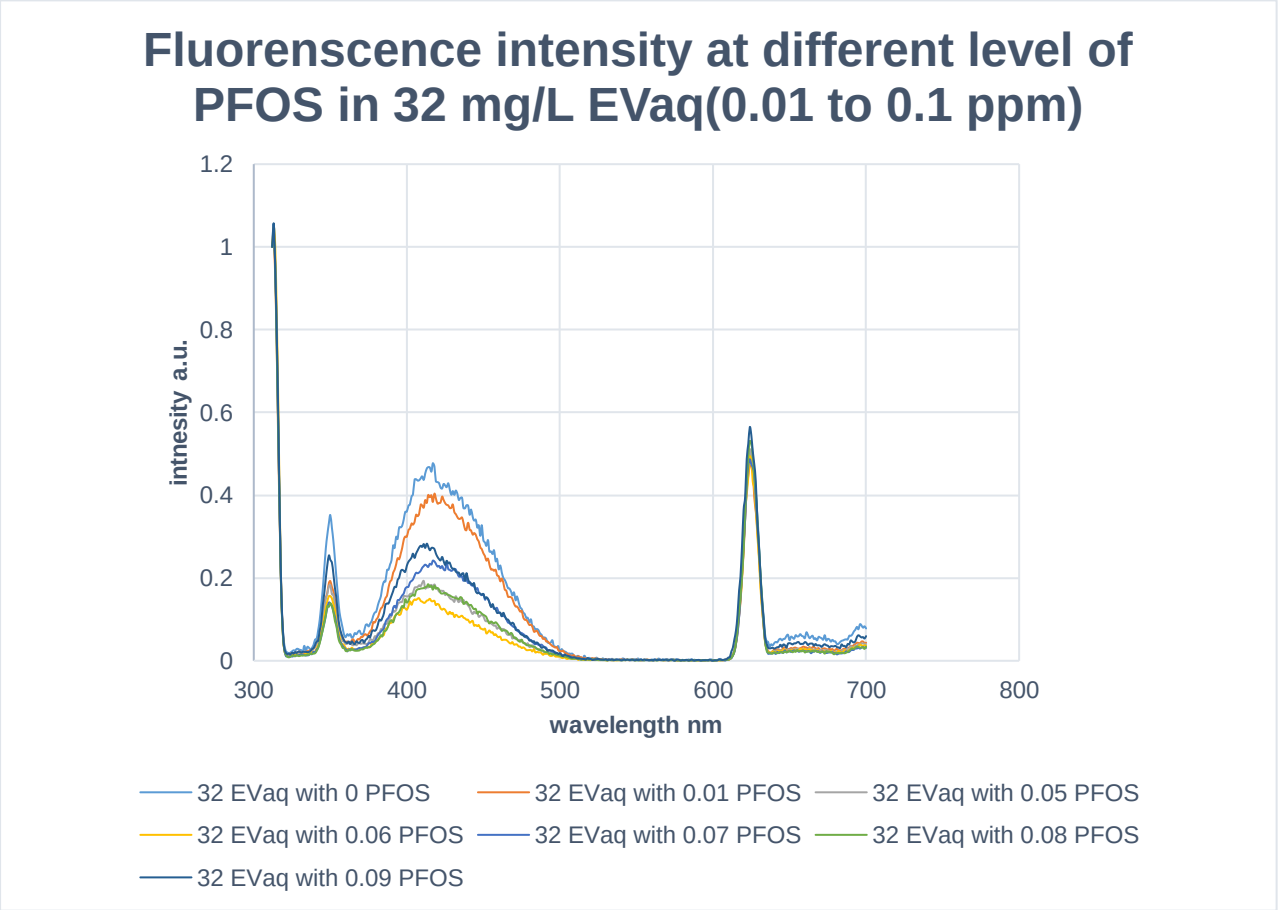

Figure 15 Fluorescence intensity at different level of PFOS in 32 mg/L EV aqueous (0.01 to 0.1 ppm)

From Figure 14 and Figure 15 above, it can be seen that results from region 0 to 0.09 ppm seem to be random, and it was impossible to establish a relationship between concentration and intensity. As much, further analysis on the data from 0 to 0.09 ppm were not carried in this research. Based on the intensity results of fluorescence peak from 0.01 to 2 ppm, the trend lines of two regions were added and shown in Figure 16 below with linear relationship equations of 0.1 ppm to 1 and 1 ppm to 2 ppm:

$y = -0.1513x + 0.3334$, $R^2 = 0.9403$ (0.1 ppm to 1 ppm)

$y = -0.0538x + 0.211$, $R^2 = 0.9633$ (1 ppm to 2 ppm)

From the relationship equations, the slope of region 0.1 ppm to 1 ppm is bigger than that of the region of 1 ppm to 2 ppm, but these two slopes are smaller than

the slope which was established in previous studies in the region 0.01 ppm to 1 ppm.

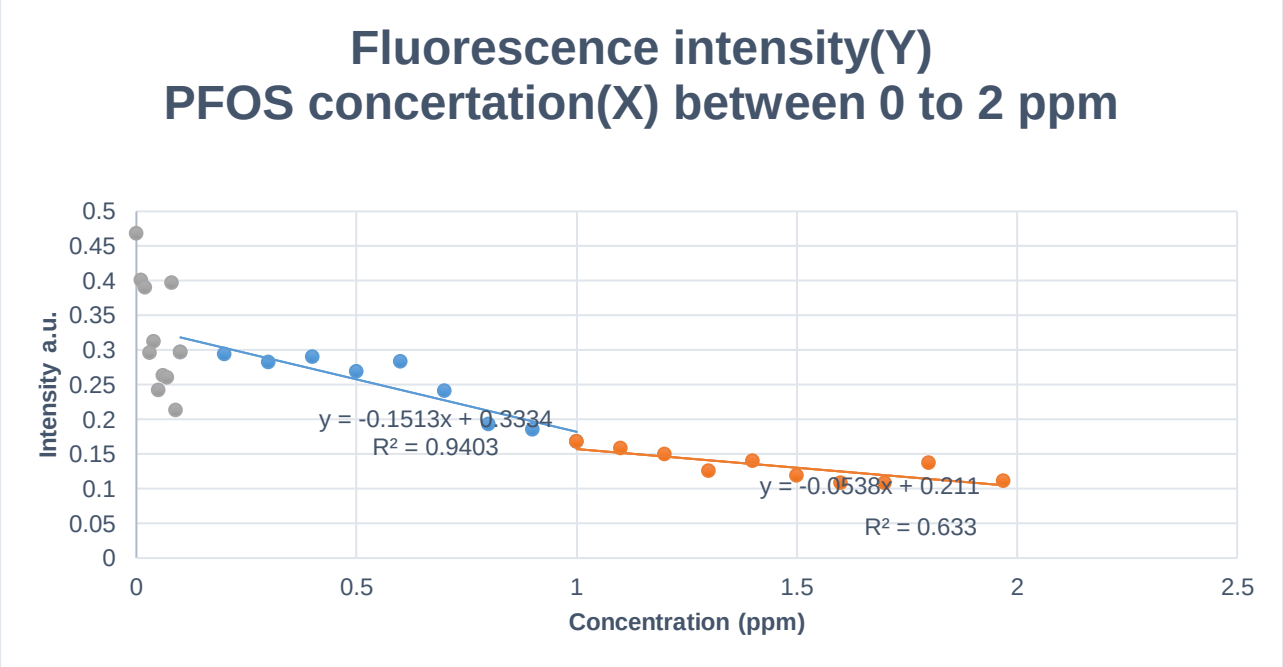

Figure 16 Fluorescence intensity Vs Concentration of PFOS

3.3 The VFD technology

To enhance the extraction efficiency, VFD technology had been used to detect low concentrations of PFOS/ PFOA, and the results have been characterised by using the previously patented astkCARE kit. The main principle of the patented method is to extract the hydrophobic ion pair of dye (ethyl violet-anionic surfactants) to the organic phase, and the colour (RGB) of the dye in the organic phase was collected by using a smartphone camera and analysed by an application. The deviation of this method compared with the standard HPLC-GL is less than 10%, as reported [29]. Several experimental parameters have been evaluated to optimise the following conditions based on the previous publication [38-40] including rotation speed, and residual time.

3.3.1 Samples preparation

The mother solution of 32 mg/ L ethyl violet and PFOS/ PFOA were prepared as above for the fluorescence method part. All concentrations of the PFOS: 5 ppb, 4 ppb, 3 ppb,

2 ppb, 1 ppb, 0.9 ppb, 0.8 ppb, 0.7 ppb, 0.6 ppb, 0.5 ppb, 0.4 ppb, 0.3 ppb, 0.2 ppb, 0.1 ppb, and a blank solution are prepared. The composition of preparing each concentration is a listed in Table 2.

Table 2 Composition of preparing each concentration

	Milli-Q water	PFOS/ PFOA	volumetric flask
5 ppb	35.79 ml	17.6 ppb * 14.21 ml	50 ml
4 ppb	38.64 ml	17.6 ppb * 11.36 ml	50 ml
3 ppb	41.48 ml	17.6 ppb * 8.52 ml	50 ml
2 ppb	44.32 ml	17.6 ppb *5.68 ml	50 ml
1 ppb	47.16 ml	17.6 ppb * 2.84 ml	50 ml
0.9 ppb	47.44 ml	17.6 ppb * 2.56 ml	50 ml
0.8 ppb	47.73 ml	17.6 ppb * 2.27 ml	50 ml
0.7 ppb	48.01 ml	17.6 ppb * 1.99 ml	50 ml
0.6 ppb	48.29 ml	17.6 ppb * 1.71 ml	50 ml
0.5 ppb	48.58 ml	17.6 ppb * 1.42 ml	50 ml
0.4 ppb	48.87 ml	17.6 ppb * 1.13 ml	50 ml
0.3 ppb	49.15 ml	17.6 ppb * 0.85 ml	50 ml
0.2 ppb	49.43 ml	17.6 ppb * 0.57 ml	50 ml
0.1 ppb	49.72 ml	17.6 ppb * 0.28 ml	50 ml
0 ppb	50 ml	0	50 ml

Each sample was transferred into their the corresponding volumetric flask, place it into ultrasound to eliminate the bubbles for 5 mins, and rest at room temperature.

3.3.2 Rotation Speed effects

To ensure the best speed for enhanced extraction efficiency, five concentrations of PFOS samples, 176 ppb, 88 ppb, 17.6 ppb, 8.8 ppb, 1.76 ppb have been chosen to react with the patented astkCARE reagent, by following the ratio recommended by the astkCARE kit, and then transferred into the syringes equipped with the syringe pump for a constant flow speed of 10 ml/ min. In previous pilot experiments, when the rotation speed is lower than 4500 rpm, the mixture of the sample and astkCARE will not fly out of the VFD container. When the speed is higher than 7500 rpm, the residual time is too short, and the generated heat will evaporate most of organic phase of the final

solution. As such, the rotation speed test region was set between 4500 to 7000rpm, as suggested for evaluation with the speed of increase every 500rpm. For more intuitive expression of the extraction efficiency(EE), the extraction efficiency has been defined in the Equation (2)

$$EE = \frac{(\,the\ nominal\ reading\ value\ of\ PFOS\ after\ VFD\,)}{(\,the\ value\ of\ real\ concentration\ prepared\,)} \quad (2)$$

where the nominal reading value of PFOS after VFD was achieved from the astkCARE kit, the real concentration value was the similar to the sample prepared. The effects of rotation speed in regards to the extraction efficiency is shown in Figure 17.

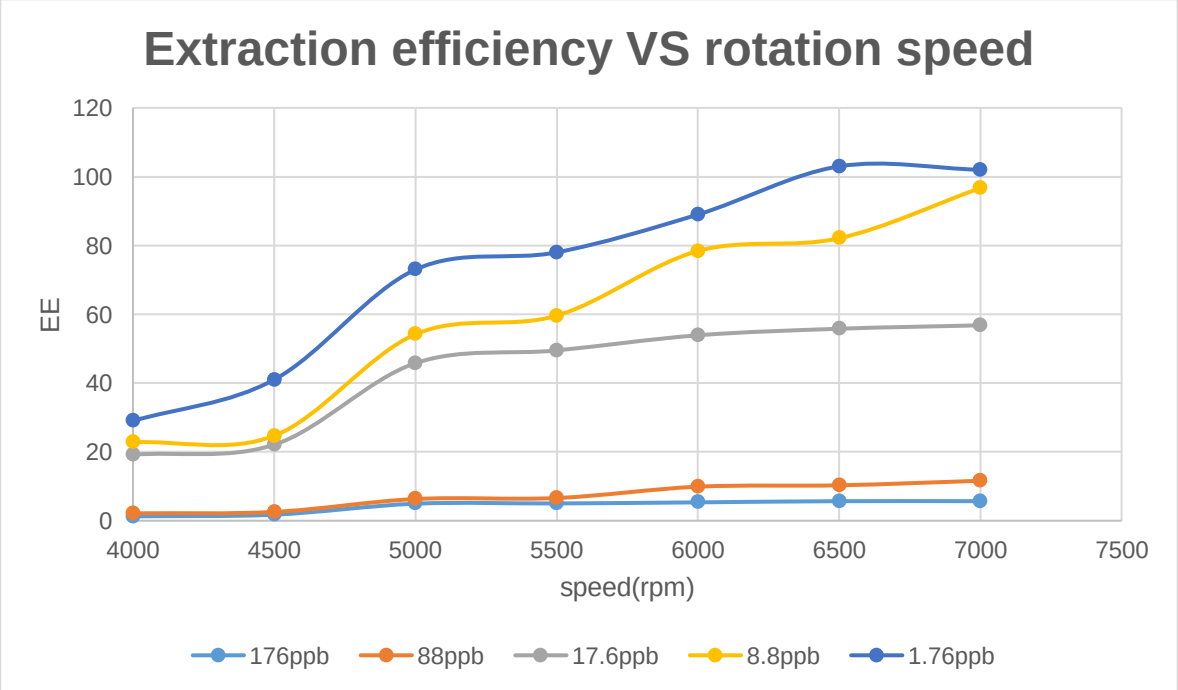

Figure 17 Extraction efficiency VS rotation speed

For the lower three concentrations, the extractions' efficiency showed a significant increase from the rotation speed of 4500 to 5000 rpm. Because the detection limit of astkCARE kit is 10ppb-1000ppb, the samples of higher concentration (176 ppb, 88 ppb) will reach the detection limit when the rotation speed is beyond 5000 rpm. When the rotation speed is over 6500 rpm, the extraction efficiency increases little and appears to fluctuate, while the residual time is shorter comparing to the other rotation speed which was harder to control and maintain the rotation speed. Considering these reasons, the best rotation speed of VFD was set at 6000 (± 50) rpm.

3.3.3 Residual time

As mentioned in the literature review, the residual time is affected by the flow rate, rotation speed and the tilt angle which determines the retained time [40]. Under the continuous mode, when the fixed rotation speed was set at 6000 (± 50) rpm, and the tilt angle was set at 45 degrees, the residual time is only dependent on the flow rate, which is controlled by the syringe pump rate, expressed by the Equation (3) below:

$$\text{Residence time} = \frac{Fluid\ Retained\ Device}{Incoming\ Fluid\ Flow\ Rate} \quad (3)$$

The two syringes used to inject the samples and the patented astkCARE reagent are 10ml each, and six flow rate speeds have been set, i.e. 0.5 ml/min, 1 ml/ min, 2.5 ml /min, 5 ml/min, 10 ml/min (manual)and 20 ml/ min(manual) with the sample concentration of 176 ppb, 88 ppb, 17.6 ppb, 8.8 ppb, and 1.76 ppb being used. The results are shown in Figure 18 below:

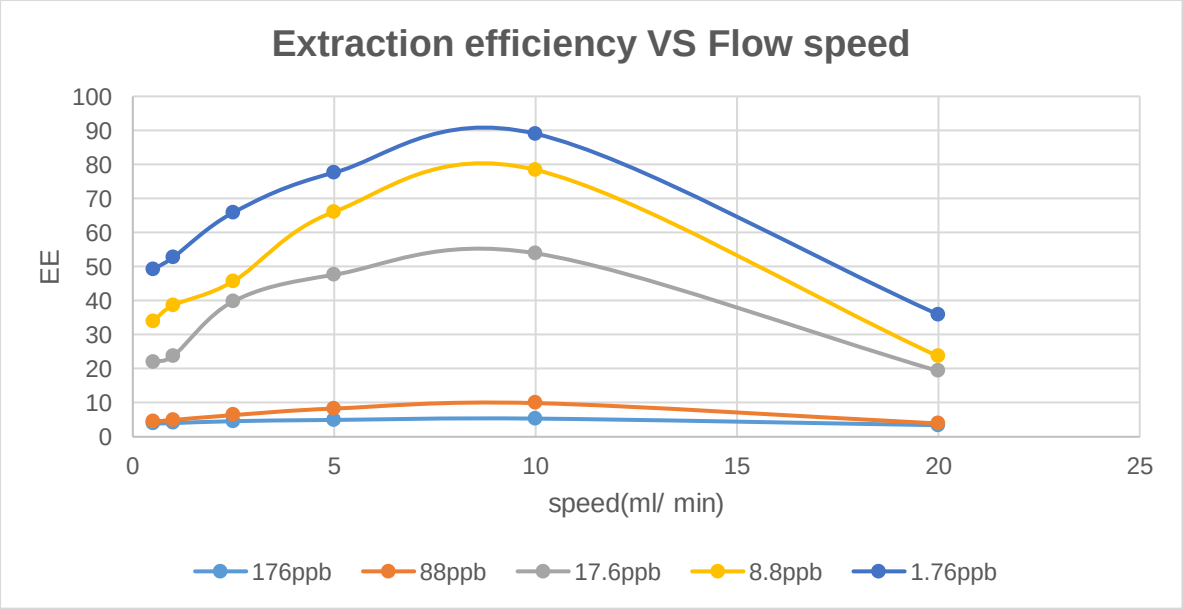

Figure 18 Extraction efficiency VS Flow speed

From the results in Figure 18, the lowest concentration sample still gets the highest extraction efficiency through the VFD pre-treatment process. Due to the limitation of

the astkCARE kit, the two samples with higher concentration i.e. 176 ppb, 88 ppb do not increase as much when compared to the other three lower concentration samples. For all of five samples, the best extraction efficiency appeared at the flow speed of around 10ml/ min, which meant that the flow should be injected into the tube at 1min for syringes of 10 ml.

3.3.4 Rest Time

As the extraction between the two phases is a dynamic process, extra time may be needed time to achieve a higher efficiency after the VFD treatment. Seven checkpoints for rest time have been chosen after the two phases flow was transferred into the container of astkCARE kit i.e. 0 min, 1 min, 5 min, 10 min, 30 min, 60 min and 24 h. The rotation speed was set at 6000 (± 50) rpm, and the residual was set at 1min. As previous experiments have shown that the lower concentration samples will appear with higher extraction efficiency after the VFD process,14 concentrations of samples were chosen from 0.1 ppb to 5 ppb for this study. Following the VFD process, the results are shown in Figure 19:

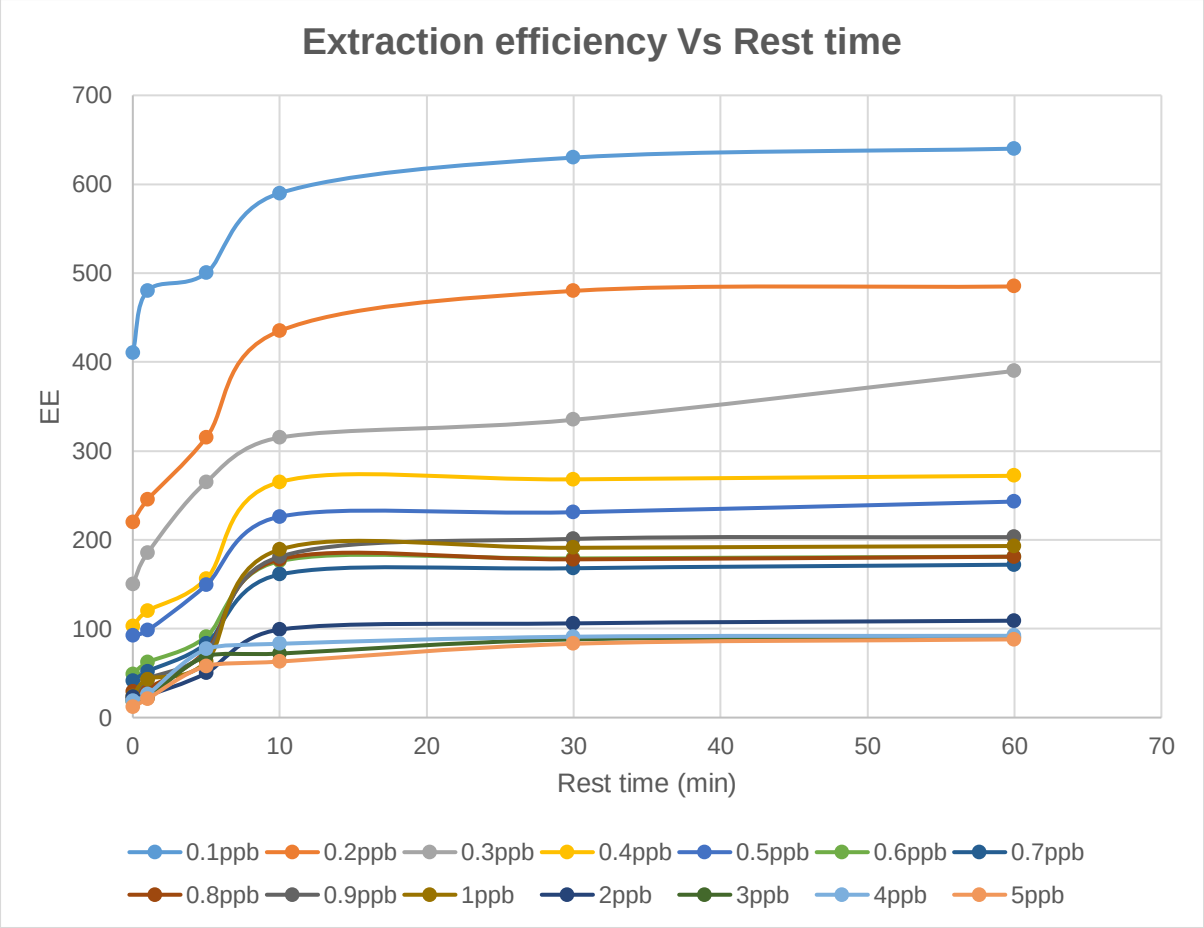

Figure 19 Extraction efficiency Vs Rest time

From the results above, the extraction efficiency of all samples had increased with an increase in the rest time, and the most significant increase appeared in the periods of 0 to 10 mins. The extraction efficiency was almost kept instant. Considering the convenience of the actual operations, the best rest time for the samples after the VFD treatment is 10 mins and the 5 mins is required to achieve acceptable results at least.

3.3.5 Cycle Time

To further explore the extraction ability of VFD and the astkCARE patent reagent, the cycle time of injecting the flow into VFD tube with water and organic phases again was tested to determine if the extraction efficiency will increase after more cycles and if so, how many cycles are needed reach the limitation of the extraction ability. Five concentrations of samples i.e. 0.1 ppb, 0.5 ppb, 1 ppb, 2 ppb and 5 ppb at 6000 rpm

rotation speed and 10 ml/ min flow speed, with the rest time between each cycle of 10 min are chosen. The results are shown in Figure 20:

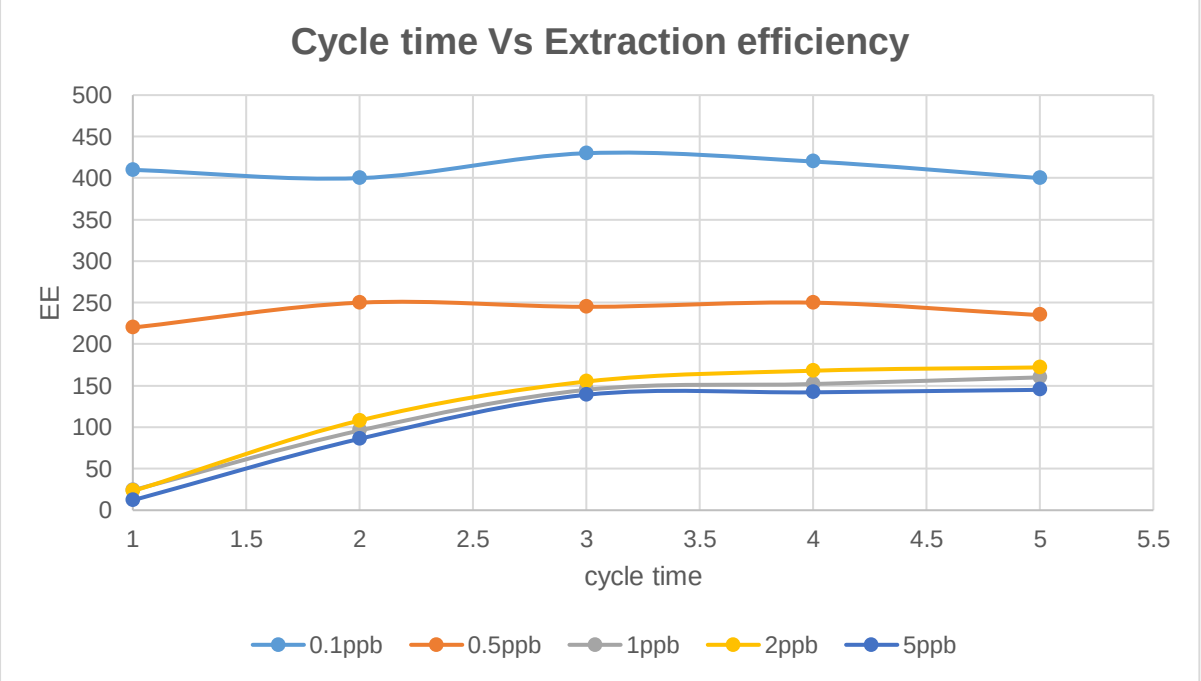

Figure 20 Cycle time Vs Extraction efficiency

From Figure 20 above, it can be seen that, the extraction efficiency for lower concentration samples were stable through several cycles of VFD, but the higher concentration samples displayed an increasing extraction efficiency after the first cycles, and the speed of increase became slower after the third cycle. As the cycling process exhausts more time, three cycles in total were chosen to enhance the extraction efficiency.

3.3.6 Best Conditions Determination and quantitative relationship building

Based on the above investigations, the best conditions for the VFD process to reach the maximum extraction efficiency can be determined with rotation speed of 6000 (± 50) rpm, 10 ml/ min flow speed, 10 min rest time and 3 cycles of pump process with that the container was being inclined 45° relatives to the horizontal position.

Under the best conditions, five concentrations samples (0.1 ppb, 0.2 ppb, 0.3 ppb, 0.4 ppb and 0.5 ppb) were chosen to establish the relationship between real

concentrations of PFOS and the value read by astkCARE kit (Nominal value of PFOS) after VFD extraction by using the optimised conditions. The results showed a linear relationships between the reading value from the astkCARE kit and the real PFOS concentration, as shown in Figure 21.

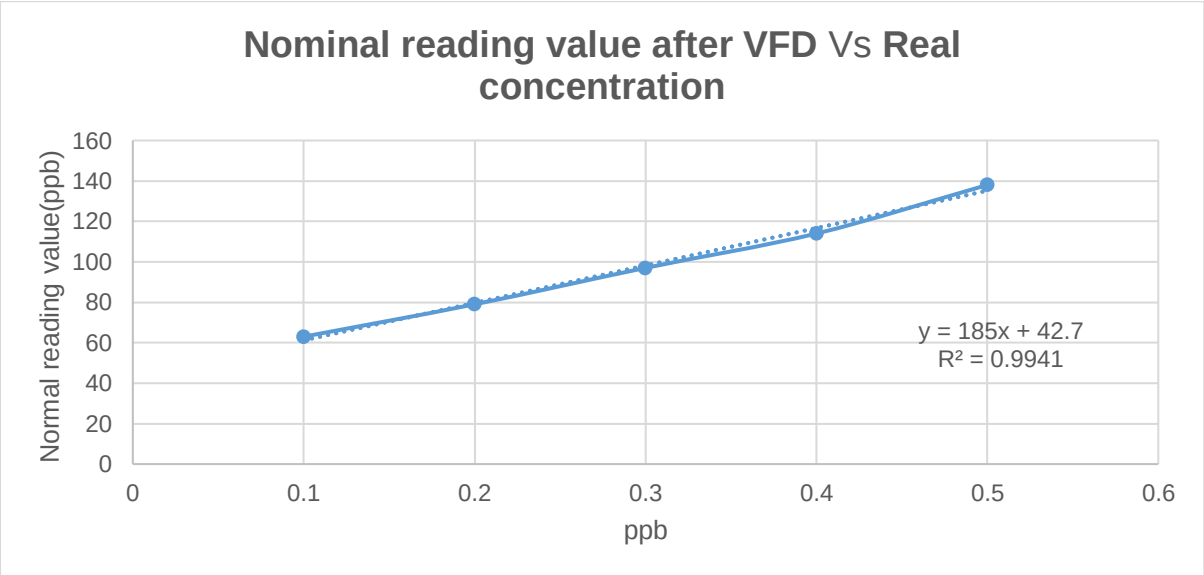

Figure 21 Nominal reading value after VFD Vs Real concentration

From Figure 21, it showed that the astkCARE kit reading value after VFD process increases as the real concentration of PFOS increase. The linear equation is: y = 185x + 42.7with the coefficient of determination R^2 = 0.994. By driving the VFD technology, the actual detection limitation of astkCARE increased to 0.1 ppb from the original limitation of 10 ppb, and the EE increase up to 600 times for the lowest concentration sample of 0.1 ppb. This result sets a solid foundation to further develop the suitable smartphone application to quantitatively report PFOS concentration on site.

CHAPTER 4: DISCUSSIONS

4.1 Fluorescence Method

In the fluorescence method, the initial fluorescence response without PFOS/ PFOA is from the EV itself. With the addition of PFOS, the combination (COM) of cationic dye (EV) and anionic surfactant (AS, PFOS) begins to form, which is insoluble in the water phase. Therefore, the fluorescence intensity appears weak as the amount of fluorescence substance (EV) is decreasing. This process could be expressed by Equation (4):

$$EV\ (aq) + AS \longrightarrow \text{COM} \qquad (4)$$

The results attained up to 2 ppm was expected to establish a relationship between fluorescence intensity and concentrations, but the corresponding intensity of PFOS concentration below 1 ppm (up to 1 ppm of PFOS) displayed a lot of randomnesses, which is the main reason for the lower concentrations results not being used for further analysis. Even though there is a linear relationship between the concentration regions over 1ppm and fluorescence intensity, the expected lower detection (sub-ppb level) limitation cannot be achieved due to the random results for concentrations under 1 ppm.

By reviewing the chemical properties of EV and AS, there are two possible reasons for the random results at the lower concentrations. Firstly, the combined ability of EV and AS is not constant, they vary depending on their concentration of themselves. When the concentrations are relatively larger, the small variation could be ignored comparing the concentration itself, but when it used to detect the lower concentrations, the difference will affect the amount of AS-EV combination, therefore resulting in the fluorescence intensity being random. Secondly, the AS and the combination of AS-EV will have slight degradation under the light. Considering the mode chosen for fluorescence spectrometer is slow, which last for 15 mins in the spectrometer for each sample testing, the amount of degradation cannot be ignored in the low concentration samples. To sum of two chemical reasons above, the random results could be

explained by the varied ability of combination between the two chemicals due to their concentration levels and degradation under illumination.

4.2 VFD Method

In the VFD method, four impact factors of the VFD processing could affect the extraction efficiency of the patented astkCARE reagent being investigated. The four impact factors are rotation speed, residual time, cycle time and rest time. The reason why rotation speed has been chosen as the first considered controllable variable is because the rotation speed is the main characteristic parameter for the main principle of the VFD as aforementioned in the literature review.

Before processing my designed experiments, a definition of the extraction efficiency was defined to evaluate the effect of VFD, which is the concentration value after the VFD process over the concentration value before the VFD process. From the results achieved, it can be concluded that the higher concentration samples appear to have lower increase in extraction efficiency when compared against the lower concentration samples, which meant that the limitation of extraction efficiency with higher concentration samples had been observed even at low rotation speed. The reason for this is probably due to the drop of higher concentration sample being easier to form the thin firm around the inner tube at even low rotation speed, therefore the increase of speed does not enhance the extraction efficiency obviously. The results from lower concentrations samples displayed more increase in extraction efficiency which could be explained by the fluid dynamic balance affected by the increase in sheer stress from inner tube friction, which results in a wider surface and greater length being generated. Wider surface would mean a better condition which provides more chance of extraction between water and organises phase. With the same extraction solution, the more extraction chance means higher efficiency. I tried some higher rotation speed over 7000 rpm to 8000 rpm were investigated for the lower concentration samples. When the rotation speed is set at 8000 rpm, the mixture flows out at a high speed was hard to control, and the extraction efficiency is a little bit lower than that of 7000 rpm. The reason for the lower extraction efficiency is that the higher rotation speed will generate more sheer via contact surface which is bigger than the maximum friction required by forming the thin firm. And the higher pressure caused by the higher rotation

speed significantly reduced the tension of film, which will reduce the size of micro-droplet according to the Gibbs thermodynamic method, therefore reducing the reaction force between two phases during the extraction process [41].

In the VFD continuous flow mode chosen, the residual time, which could affect the reaction time, is only determined by three factors: input flow speed, rotation speed and tilt angle [37]. The rotation speed and tilt angle have been fixed, therefor the residual time is only controlled by the input flow speed. As the input flow was though the stainless-steel tube, the entry form of the mixture of samples and the astkCREA reagent will be determined by the speed of input flow, which means when the speed of the input flow is high, the mixture will flow in through the stainless-steel tube as a water slip; when the speed of the input flow is low, the mixture will flow in to the tube as drops. As previous researches results shown, the form of the input flow will affect the forming efficiency of the inner firm which is an important factor in the reaction efficiency. In general, the best input flow speed is that when the amount of every input flow will form the suitable inner firm thickness for the extraction of the process. In this experiment, the exact thickness of best conditions for the extraction process is not measured, but the input flow speed of 10 ml/ min did appear the best result which means this speed is the best condition for the inner form thickness.

As we know, the extraction process is a dynamic process from initial mixing to even after the VFD process. The VFD technology makes the extraction process much faster when compared to the conventional extraction method including the rest period. When the sample mixture flows out of the VFD cover, the two phases' separation appears almost instantly, but the dynamic micro process is still going on. Theoretically, as the time goes on, the extraction process will be much more fully which is proved in my experiment. The results did not show any increase after 10 mins for all samples, and even most of the samples show little increase after 5 mins. The results indicated that most extraction ending point will appear in 5 mins after VFD treatment which could be explained by the dynamic process not affecting the reading value after 5 min, and for more accurate results, the rest time could be extended to 10 min.

Finally, more cycling time was introduced to this designed experiment for a better result. As mentioned above, the residual time is an important factor for improving the extraction efficiency. Under the best rotation speed, the residual time will be a fixed

value. Therefore, the output flow mixture was immediately injected into the tube with the same input speed to further enhance the extraction efficiency. The results have showed that after three cycles of the VFD treatment, the best extraction efficiency will be achieved, which means for the fixed ratio of sample and astkCARE reagent, the astkCARE limitation of extraction ability appears after three VFD processes.

CHAPTER 5. FEATURE WORK

The real sample was obtained from Royal Australia Air Force (RAAF) base runoff-Helps Rd, Salisbury, SA, which contained 0.1 ppb PFOS detecting as detected by HPLC-MS conducted by Dr Justin Chalker from Institute for Nanoscale Science and Technology Flinders University. Firstly, the sample is boiled for 1-2 min and cooled to room temperature, and then the Millex-GS filter (0.22 µm, limit 150 psi, 4mm diameter, Merck Millipore Ltd) was then used to eliminate the organic particles. By applying the best VFD conditions as mentioned above, results from rest time 0min to 10 min was recorded and shown in Figure 22.

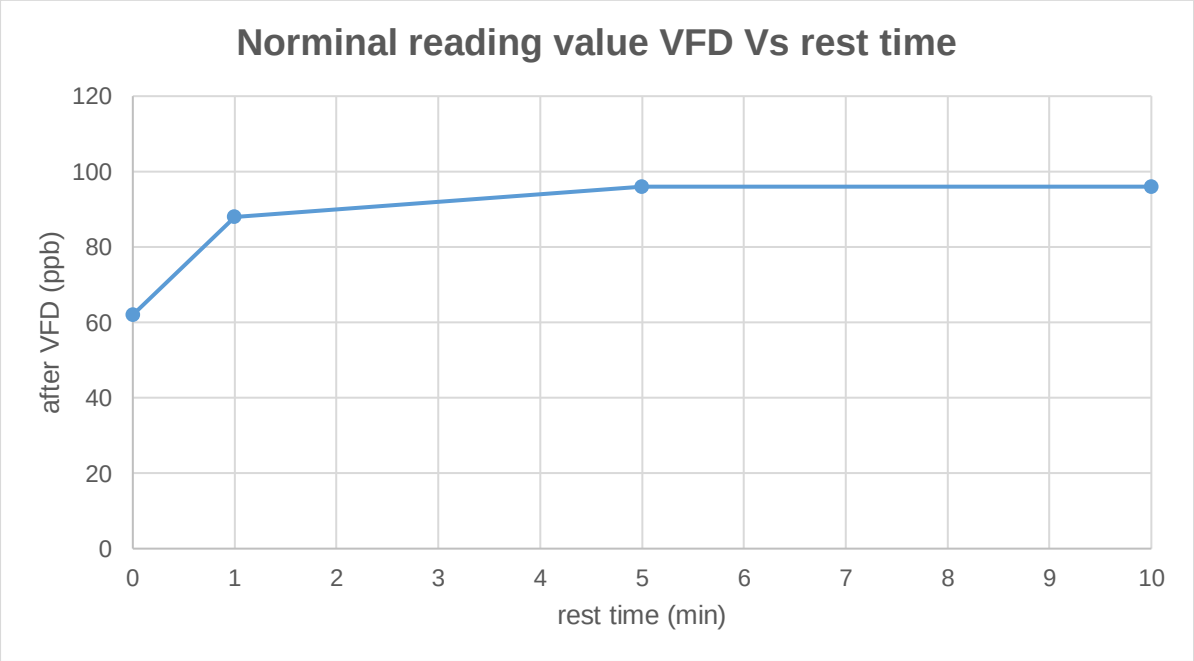

Figure 22 Nominal reading value Vs rest time

The results showed that the maximum concentration value appears within the 5 mins after the best conditions of VFD process, which is bigger than the result from as the prepared sample (0.1 ppb) in the milli-Q water. According to the relationship established above, the concentration should be 0.296 ppb, which is far greater than the actual value from attained the HPLC-MS method.

The reading value after VFD is larger than the expected value could be explained by the facts that there are other organic or ironic combined with the cationic dye. Therefore, the first job in the future work is to eliminate the effects of other substances obtained in the real sample by simple methods such as boiling with H_2O_2. The other part that needs to be improved is the revision of the validation method for the application in the smartphone. The original application validation method was established on the conventional extraction method, which has significant deviation comparing the nominal reading values after VFD pre-treatment and the real concentration value of PFOS/ PFOA. The future job will be based on the HPLS-MS results of the standard solution to generate a calibration curve as the inventor of astkCARE kit did following the instruction suggested the US EPA.

The astkCARE kit itself also needs to be opyimised. On one hand, the current size and weight of astkCARE has the potential to reduce significantly. The materials of the holder for the astkCARE kit is aluminium which is lighter than other common mental but considering the purpose of this holding is just to provide a container for samples and led light, it could be replaced by other plastic-like UHMWPE. The size of the holder could be smaller in length and diameter as well, which will not affect the function of the astkCARE kit after redesign the location and the light path of the LED lights. Further optimise should be based on reduce of the size of the sample container, which requires less space of the holder and smaller LED luminance with less electric power supply. The expected goal of the astkCARE kit is small and light enough to carry out, similar to a cell-phone and the connection and fixation method of the smartphone should be akin to the plug which is suitable for the common size of a smartphone and easy to twist.

For the VFD device part, even though it is compact enough considering its control function and high rotation speed, two features will be designed in the next stage work. Inspired by the novel work by Bhamla, M. Saad [42] reported by his Nature Biomedical Engineering publication, the hand-powered high-speed rotation design will be introduced in the future to improve the VFD rotation part, which is shown in Figure 23.

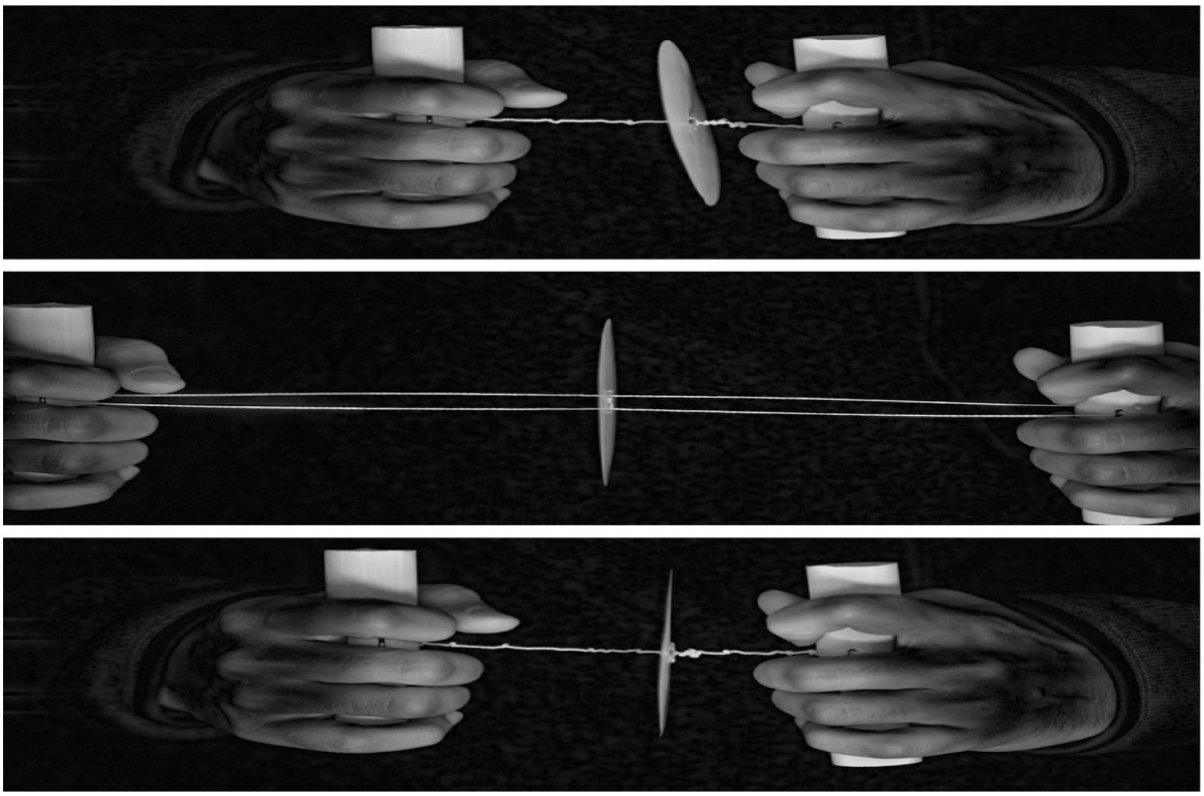

Figure 23 Hand-powered ultralow-cost paper centrifuge [42]

As the size of the sample container of the holder component reduced part, the size of the sample tube will be reduced as well. The expected solution will be that the sample container will be used in the rotation process and the transfer process will be ignored, which would mean that the sample will be injected into a small container and directly placed into the modified astkCARE kit for capturing after the rotation process.

The astkCARE patented reagent will be another improvement in the future work. When the rotation process was driven more than once, the loss of reagent during the transfer process is unavoidable, which is an important factor affecting the reproducibility and the precision of the experiment. An idea is to design a recycle device connecting the input and output flow with a control switch. This idea is easy to accomplish but will result in the increase in compounds of the device and might limit its onsite application. Another idea is trying to identify an alternative ratio of the patented reagent to increase the extraction ability and reduce the cycle time for the VFD process. The expected result will be that three extractions ending point appears within one cycle under best conditions.

To summary, in the next stage, future work will focus on the three parts: reducing the size of astkCARE kit; making the VFD rotation part powerless and more portable; a more efficiency extraction reagent or a device to achieve the rotation cycle easily without reagent lose. The expected goal is a device which could be handheld and easily operated, short time consuming with high accuracy and detecting limitation.

CHAPTER 6. CONCLUSIONS

Throughout this work of two semesters on my project of the thesis, two methods including the fluorescence method and the VFD treatment method were investigated to enhanced detection limitat of PFOS/ PFOA with an existing portable and relatively accurate device. The fluorescence method was expected to achieve a low detecting limitation by establishing the relationship between EV solution fluorescence intensity and the concentration of PFOS/ PFOA. No lower concentrations could be detected and measured. The VFD pre-treatment was expected to shorten the extraction time before the measurement being conducted by the current astkCARE kit. After the optimising the VFD conditions, the extraction efficiency was enhanced significantly when compared against the original concentration. The relationship between the lowest 5 concentrations and the reading value was initially established. Based on the relationship, a sample obtained onsite was measured using the optimised conditions and a deviated result was attained.

The results of this project displayed significant advances when compared to the original astkCARE kit measurement which approaches the requirement of the current strictest standard for drinking water and presents further potential to portably detect PFOS/ PFOA concentration of sub-ppb level on site.

In the future work, the compact and powerless VFD device will be redesigned and remodelled. The validation relationship will be established in the application of smartphones based on the reading value after VFD conditions and the real concentration to detect an accurate ppt concentration.

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