

DEVELOPMENT OF *IN-SITU* SUSPENDED AGGREGATE
MICROEXTRACTION AND MULTI STEPS STACKING-CAPILLARY
ELECTROPHORESIS OF TARGETED MICROPOLLUTANTS

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy

Faculty of Science
Universiti Teknologi Malaysia

MAY 2022

ACKNOWLEDGMENT

I would like to express my heart full of gratitude to anyone in contact intentionally or unintentionally towards the preparing of this thesis. Upon all the contribution and help that I receive in finishing this study. Firstly I would like to thank Allah S.W.T for giving me the blessings throughout my whole life. The ups and down in making this study to finish line was a hard process that require high determination and load of patience. I am very thankful towards my supervisor Associate Profesor Dr Jafariah Jaafar for her support, guidance and encouragement. I would like to express my appreciation to my co-supervisors, Associate Professor Dr Zaiton Abdul Majid and Professor Dr Azmi Aris for their advice and motivation. Without their guide and continued support the thesis will not be presented here today.

I am also appreciative to two Research Grants 12H03 and 4L810 in funding my studies. I am also indebted to MyBrain 15 for funding my University fees. I would like to express my gratefulness to Universiti Teknologi Malaysia (UTM) for providing complete facilities requirement for this study. I want to treasure my sincere experience with all the staff in University Industry Research Laboratory (UURL) especially Mr Amirul Amin Khir Anuar for providing all my requirement on operating the Capillary Electrophoresis instrument. I also thanked the staff in Chemistry Department and IPASA for all the material support towards my studies.

My sincere appreciation to fellow friends from Zeolite lab, IPASA, KDSE, Research Alliance and PPA Faculty Science team in helping and continuously supporting me. Their thought, tips and emotional support will be remembered for the rest of my life. I am also grateful to my siblings and family members, without them I couldn't be where I am now today.

ABSTRACT

Micro-pollutants are chemical and biological contaminants which are present in trace amounts in water bodies. A new sample pre-concentration technique known as *in-situ* suspended aggregate micro-extraction (iSAME) was used for the extraction of targeted environmental micro-pollutant in river waters samples. Extracts of indomethacin, diclofenac and ibuprofen by iSAME were analyzed using the multi-step field enhancement sample injection (FESI)-sweeping-micelle to solvent stacking (MSS) capillary electrophoresis (CE). The iSAME was optimized in terms of surfactants selection and concentration, temperature and extraction time. The FESI was carried out by creating a buffer zone to increase the sample load to enhance the signal. Sample was introduced into a 56 cm, 1% polydiallyldimethyl ammonium chloride (PDDAC) coated capillary at -10 kV, and the sweeping used 15 mM hexadecyltrimethyl ammonium bromide (CTAB) as surfactant injected at 10 kV and MSS with 60% methanol injected at 50 mbar for 6 second. A combined surfactant of 10 mM CTAB with 25 mM 5-sulfosalicylic acid dihydrate (SSA) was found to give iSAME extraction efficiencies of 73.16, 77.93, and 83.81% for ibuprofen, diclofenac and indomethacin, respectively. Reversal of electro-osmotic flow (EOF) and migration order were recorded with the coated capillary. The limit of detection ranged from 0.011 to 1.148 $\mu\text{g/mL}$ for standard solutions in FESI-Sweep-MSS. The sensitivity enhancement factors (SEF) of 727-898 were achieved by FESI-Sweep-MSS. The developed method was successfully applied for the detection of targeted pharmaceuticals in river water at four sampling stations. The quantification for the spike river water sample was performed by standard calibration method. The recoveries in spiked river water sample ranged from 13% to 79 % for four assays with RSD of 0.81, 0.96 and 1.1 for ibuprofen, indomethacin, and diclofenac, respectively. The iSAME and multi-step FESI-Sweep-MSS was successfully developed for the analysis of standard pharmaceuticals solution. The low recoveries of analytes in river water sample could be attributed to the high suspended solid present in the real river water sample which interfered with iSAME steps.

ABSTRAK

Pencemar mikro ialah bahan pencemar kimia dan biologi yang terdapat dalam jumlah surih di dalam badan air. Teknik pra-pemekatan baharu yang dikenali sebagai pengekstrakan agregat mikro setempat terampai *in-situ* (iSAME) telah digunakan untuk pengekstrakan bahan pencemar mikro sasaran di dalam sampel air sungai. Ekstrak indometasin, diklofenak dan ibuprofen dengan iSAME dianalisis menggunakan elektroforesis kapilari (CE) suntikan sampel penambahbaikan lapangan pelbagai langkah (FESI) penyapuan misel kepada penyusunan pelarut (MSS). Teknik iSAME dioptimumkan dari segi pemilihan dan kepekatan surfaktan, suhu dan masa pengekstrakan. FESI dilakukan dengan mewujudkan zon penampakan untuk meningkatkan muatan sampel untuk peningkatan isyarat. Sampel disuntik ke dalam kapilari 56 cm yang disaluti 1% polidialildimetil amonium klorida (PDDAC) pada -10 kV, sementara sapuan menggunakan heksadesiltrimetil ammonium bromida (CTAB) 15 mM sebagai surfaktan disuntik pada 10 kV dan MSS menggunakan 60% metanol disuntik pada 50 mbar selama 6 saat. Gabungan surfaktan 10 mM CTAB dengan 25 mM asid 5-sulfosalisilik berhidrat (SSA) didapati mencapai kecekapan pengekstrakan iSAME 73.16, 77.93, dan 83.81%, masing-masing untuk ibuprofen, diklofenak dan indometasin. Pembalikan aliran elektro-osmosis (EOF) dan susunan migrasi telah dirakamkan dengan kapilari yang bersalut. Had pengesanan untuk FESI-sapuan-MSS menggunakan larutan piawai adalah dalam julat 0.011 hingga 1.148 $\mu\text{g/mL}$. Faktor peningkatan kepekaan (SEF) 727-898 telah tercapai untuk FESI-sapuan-MSS. Kaedah yang dibangunkan telah berjaya digunakan untuk pengesanan farmaseutikal terpilih di dalam air sungai di empat lokasi. Kuantifikasi sampel air sungai telah dilakukan dengan kaedah penentukuran piawai. Perolehan semula di dalam sampel pakuan air sungai ialah masing-masing pada julat 13 to 79% dan RSD 0.81, 0.96 and 1.1 untuk ibuprofen, indometasin dan diklofenak. iSAME dan FESI-sapuan-MSS pelbagai langkah ini telah berjaya dibangunkan untuk analisis larutan piawai farmaseutikal. Perolehan semula analit yang rendah di dalam sampel air sungai boleh dikaitkan dengan kandungan pepejal terampai yang tinggi di dalam sampel air sungai yang mengganggu langkah iSAME.

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LIST OF ABBREVIATIONS

DOE	-	Department Of Environment
WQI	-	Water Quality Index
BOD	-	Biochemical Oxygen Demand
COD	-	Chemical Oxygen Demand
DO	-	Dissolve Oxygen
SS	-	Suspended Solid
NOM	-	Natural Organic Matter
EDC	-	Endocrine Disrupting Chemicals
PPCP	-	pesticides and other bioactive chemicals
CEC	-	Chemical of Emerging Concern
TP	-	Transformation Products
WWTP	-	Waste Water Treatment Plant
WHO	-	World Health Organization
SPE	-	Solid Phase Extraction
CE	-	Capillary Electrophoresis
iSAME	-	in-situ Suspended Aggregate Micro Extraction
HPLC	-	High Performance Liquid Chromatography
NSAID	-	Non Steroidal Anti-Inflammatory Drug
LLE	-	Liquid-Liquid Extraction
SPME	-	Solid Phase Micro Extraction
DLLME	-	Dispersive Liquid-Liquid Micro Extraction
SBSE	-	Stir Bar Sorptive Extraction
SDME	-	Single-Drop Micro Extraction
HF-LPME	-	Hollow- Fiber Liquid Phase Micro Extraction
GC	-	Gas Chromatography
RSD	-	Relative Standard Deviation
BGE	-	Background Electrolyte
EOF	-	Electroosmotic Flow
LOD	-	Lower Detection Limit
QL	-	Quantitation Limit

MEKC	-	Micellar Electro Kinetic Chromatography
CEC	-	Capillary Electrokinetic Chromatography
CZE	-	Capillary Zone Electrophoresis
ITP	-	Isotachopheresis
CGE	-	Capillary Gel Electrophoresis
IEF	-	Isoelectric Focusing
UV	-	Ultra-Violet
NaOH	-	Sodium Hydroxide
CTAB	-	Hexadecyltrimethyl ammonium bromide
SSA	-	5-Sulfosalicylic Acid Dihydrate
TIRON	-	4,5-Dihydroxy-1,3-Benzenedisulfonic acid disodium salt monohydrate
CEMS	-	Capillary Electrophoresis Mass Spectroscopy
PVP	-	poly(vinylpyrrolidone)
MS	-	Mass Spectrometer
LIF	-	Laser-Induced Fluorescence
CCD	-	Contactless conductivity Detector
DAD	-	Diode Array Detector
PDDAC	-	Poly(diallyldimethyl ammonium chloride)

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CHAPTER 1

INTRODUCTION

1.1 Extraction of micro-pollutants

In recent years, micro-pollutants of emerging concern are broadly distributed throughout the environmental matrices because of various industrial practices and man-made activities. Various extraction techniques have been developed throughout the years, serving as a principal method for isolating compounds from matrices in real samples. The methods have proven to be very helpful as a recovery method of many components in a sample matrix. Extraction is a method of separation on the basis of their solubility in two different immiscible liquid which giving the desired compound either in the form of solute or residue. Extraction also move compound from liquid to others, so the compound can be easily concentrated. Extraction technique can be in various type and extracted compound can also in various form. This is to enable selective removal of components in mixture. Thus, in this research the technique chosen was extraction by using surfactant to capture analyte in a suspended aggregate form. Surfactant belongs to amphiphiles group i.e., a molecule having both hydrophobic and hydrophilic components. Hydrophobic generally referred as tail group while hydrophilic as a head (Texter, 1999). The selection of cationic surfactant such as cetyltrimethylammonium bromide (CTAB) incorporated with derivatives as counter-anion were based on the aggregation of long chain trimethylammonium surfactants with benzene sulfonic anionic counter part (Benede. *et al.*, 2015).

The environmental concern and the green chemistry approach to decrease organic solvent usage in preparation of samples in trace analysis is fostering the search for alternative methods. Nowadays, analytical chemists look to reduce the amounts of solvents and chemicals used in analytical experiments, so miniaturization of conventional extraction methods is recommended (Melnyk *et al.*, 2014). A variety of liquid-phase micro-extraction methods, which, compared with conventional methods, are simpler, faster, and inexpensive, and involve more environmental-friendly sample-preparation techniques are being explored (Gómez *et al.*, 2010; Yazdi, 2011). The liquid–liquid phase separation of surfactants, induced by environmental conditions, temperature electrolytes and pH, has been largely used in analytical extraction and concentration schemes (Gómez *et al.*, 2010). The surfactant-rich phase is a nano-structured liquid, recently named as supramolecular solvent, generated from the amphiphiles through a sequential self-assembly process occurring on molecular and nano-scales (Baghdadi and Shemirani, 2009).

micro-pollutants are not included as pollutant in WHO list due to its low detection limit (WHO, 2021). Common practice in Malaysia is to classify water pollution in class I, II, III, IV, and V based on water quality index (WQI). The common parameters in WQI included temperature, turbidity, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammonical Nitrogen, Acidic and Alkaline (pH), Dissolved Oxygen (DO), Fecal coliforms (FC), Total Suspended Solid (TSS) and Total Dissolve Solid (TDS) (Arman *et al.*, 2013, Uddin *et al.*, 2021). Hence, WQI of all models does not consider any toxic or radioactive constituents. However, recent models such as Oregon Index, Dojildo Index, Liou Index, Almeida Index and West-Java Index recommended to include toxins (detergent, phenol), pesticide, and trace variable (Pb, Cd, Zn, Hg, Mn, Fe, etc.) for evaluating quality in water body. micro-pollutant such as pharmaceutical and its derivative has not yet to be included in any parameters of WQI indexes all over the world (Uddin *et al.*, 2021). Thus, to analyse the micro-pollutant, analytical method with correspondingly low detection limit are needed (Farré *et al.*, 2008).

Recent technologies have improved the ability to detect and quantify a variety of low concentration chemical pollutants in aquatic environment (Kong *et al.*, 2015). New technologies of extraction were constantly having improvement and enhancement. Sample preparation such as extraction were frequently performed to make sample properties compatible with the analytical instrument or in this research study, sample preparation was performed to pre-concentrate the analyte. The enhancement of extraction in recent studies included micro-extraction such as liquid phase micro-extraction (LPME), solidification of floating organic droplet micro-extraction (SFODME), and micro-extraction using surfactant (Kannouma *et al.*, 2021). The micro-extraction techniques not only offer the ability to separate the target analytes from the sample solution, but also reduce, control or even eliminate the interferences originally present (Anderson and Yao, 2009; Baghdadi and Shemirani, 2009; Benedé *et al.*, 2015).

1.2 Online Pre-concentration

Online preconcentration method using capillary electrophoresis (CE) was applied to enhance the signal of low concentration analytes. Online preconcentration of FESI-sweep-MSS was chosen as the preconcentration method. CE is a powerful separation technique, however due to small capillary sample introduced in the short length is practically lower and make it difficult to detect low analyte concentration (Rabanés *et al.*, 2012). FESI can be performed by establishing their contradiction in term of conductivity between sample and background electrolyte. FESI can introduced large amount of sample due to electrokinetic injection. FESI boundary was created when sample solution was injected in a low conductivity circumstance (Thang *et al.*, 2016, Chu *et al.*, 2018). Sweeping was invented to further enhance band narrowing and to improve the focusing of large volume sample in CZE (Aranas *et al.*, 2009). Sweeping was introduced when positive charged micelles injected at positive polarity and swept the FESI stacked anionic electrolyte (Grochocki *et al.*, 2016). In MSS, cationic surfactant is necessary in the process of stacking the anion analyte because the charge of analyte should be opposite to that of the micelle. A boundary created between methanol and cationic surfactant, CTAB, will transport the charged analyte (anion) by oppositely charged micelles (cation) towards the solvent

rich zone where interaction between analyte and micelle will significantly reduce, leading to analyte focussing (Grochocki *et al.*, 2015, Chu *et al.*, 2018).

1.3 Water Pollution

Water pollution is one of the main concern these days with a threat to human and environmental future development. The Department of Environment Malaysia (DOE) had established river water monitoring since 1978 to establish baselines and detect water quality changes in river water quality. Cleanliness status of a river is confirmed using Water Quality Index (WQI) and also Interim National Water Quality Security (INWQS). The WQI listed six main parameters which are Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammoniacal nitrogen (NH₃-N), pH, Dissolved Oxygen (DO) and Suspended Solid (SS). The WQI serves as a basis for environmental assessment of a watercourse in relation to pollution load categorization and designation of classes of beneficial uses as provided for under the National Water Quality Standards for Malaysia (NWQS). (Malaysia Environmental Quality Report 2006, Department of Environment, Ministry of Natural Resources and Environment, Malaysia) Other parameters such as heavy metal and bacteria are based on the needs. Heavy metals such as arsenic, cadmium and mercury can also be detected in water. Unfortunately these water quality index has not included organic or pharmaceuticals micro-pollutants (Al-Odaini *et al.*, 2011; Geissen *et al.*, 2015).

micro-pollutants are small, persistent and biologically active substances that are found in aquatic environments all over the world and can have negative effects on plants, animals and humans. The European Union recently adopted a ‘watch list’ of potential priority substances, including pesticides, pharmaceuticals and personal care products that need to be monitored to determine their environmental risk (Barbosa. *et al.*, 2016). The worrying fact of these micro-pollutants is that they are finding their way into water bodies and eventually into drinking water supplies. Some of these chemicals are likely to have potential hazards on human health if given constant exposure, even at very low level. Additionally, it is now becoming clear that the

situation in natural waters is considerably more complex, mixtures of substances can produce additive effects, and new stressors such as increased water temperatures or higher level of ultraviolet radiation associated with climate change, can exacerbate the situation (Jin and Peldszus, 2012)

With an increasing scientific knowledge and technological advancement, people are more concern about the widespread distribution of environmentally micro-pollutants. The micro-pollutants possess serious ecological threats and potential risks to human health and aquatic life, even at very low concentrations and pose a significant challenge to policy regulators, engineers, and scientific community. micro-pollutants that are of interest in water industry include natural organic matter (NOM), endocrine disrupting chemicals (EDC), pharmaceuticals and antibiotics, polymers, pesticides and other bioactive chemicals (PPCPs). While some of these chemicals are present in water naturally, many are synthesized for human daily needs which include industrial chemicals, pesticides and biocides, medicines, cleaning agents, flame retardants for furniture and plastics, termed as chemical of emerging concern (CEC). The term CEC mainly refers to those contaminants which no regulations currently established that requires monitoring or public reporting of their presence in the water supply or wastewater discharges. Pharmaceuticals, for example, newly recognized class of environmental pollutants, are becoming increasingly problematic contaminants of either surface water or ground water around industrial and residential communities (Kümmerer, 2009). The presence of pharmaceuticals was first identified in surface and wastewaters in the United States and Europe in 1960s (Stumm-Zollinger and Fair 1965).

Group of micro-pollutant was detected in an aquatic environments as reported by Carlos *et al.*, 2012. The removal of emerging pollutant such as clofibre acid, amoxicilin, acetamiprid, acetaminophen, carbamazepine and caffein detected on surface water were studied by using photochemical method (Carlos *et al.*, 2012). Another class of micro-pollutants are drug of abuse, steroid, industrial additive and gasoline additive detected in sewage and surface water which subsequently pollute the aquatic environment. (Farré *et al.*, 2008). Disinfection such as by chlorination or UV irradiation is also one of the source of micro-pollutant discharge.

Chlorination by-product resulted from reaction between organic and inorganic matter in water which originated from additional contaminant, biological fluid and personal product. Report showed that this kind of micro-pollutant in aquatic environment was a growing concern largely because of its unknown effect (Kong *et al.*, 2015). More than 700 micro-pollutants, their metabolites and transformation products (TPs) are listed in European aquatic environment and the potential impact is urgently required (Geissen *et al.*, 2015).

Throughout these years, many research are convergent toward micro-pollutants, thus it become one of the priority research areas of major organization (World Health Organization; the agency for environment protection, the European Commission). There were some recorded cases where reusing treated wastewater had been practiced for the last 30 years in Gran Canaria (Estevez *et al.*, 2012). The reclaimed water (treated water) had been used in the purpose of irrigation such as watering plant or water sprinkler on the golf course. Therefore, sorption and degradation of soil or surface run-off causing the present of micro-pollutant in water bodies. micro-pollutants can be transported and distributed via various routes to reach aquatic environment, for example from waste water treatment plant (WWTP) and also surface run-off (Farré *et al.*, 2008). According to Al-Odaini *et al* (2013), pharmaceutical micro-pollutants has not received enough attention for water treatment due to lack of monitoring as it is not listed as pollutants in WHO Guideline for drinking water quality (Al-Odaini *et al.*, 2013).

1.4 Statement of problem

The present technique used by water quality laboratories is extraction by SPE cartridges which can often be clogged by water samples with high turbidity and thus takes a long time to process the water before analysis. To overcome this problem, a new micro-extraction technique was explored using *in-situ* suspended aggregate micro-extraction (iSAME). The technique should be able to be performed on raw water samples without the need for water sample pre-filtration, and it needs small volume of sample and thus use less eluting solvent.

The separation and detection of micro-pollutants can be achieved using capillary electrophoresis. However, UV-visible detector has some limitation in detection at low concentration. Thus, to enhance the detection sensitivity, a multi-step stacking involving field enhanced sample injection (FESI), sweeping and micelle to solvent stacking (MSS) was introduced. The detection of new emerging micro-pollutants can be achieved by an efficient extraction technique combining off-line micro-extraction and multi-step on-line pre-concentration. Hence, this study will be conducted to explore the use of micro-extraction and multi-step CE for the analysis of selected pharmaceutical micro-pollutants in river water.

1.5 Significance of Research

This study shall focus on the analysis of pharmaceuticals namely ibuprofen, indomethacin, and diclofenac as micro-pollutants in environmental river water samples. In this study, the extraction of these pharmaceuticals from river water using an *in-situ* suspended aggregate micro-extraction (iSAME) can provide the alternative of conventional SPE extraction method. The multi-step stacking of CE can offer an on-line preconcentration needed to increase the detection sensitivity to ng/mL concentration.

1.6 Hypothesis

The injection of low volumes in CE with UV-Vis detection is a disadvantage for the concentration sensitivity. Sensitive detection can decrease the detection limits, which is important for trace-level analysis. CE with online pre-concentration technique can compensate and enhance the signal.

The hypotheses of the study are:

1. The offline preconcentration by using iSAME can improve the extraction efficiency.

2. The extracted samples analysis by online FESI-Sweep-MSS will enhance the detection sensitivity of the CE.

1.7 Objective

The objectives of the study are:

1. To analyze trace level of pharmaceutical such as ibuprofen, indomethacin, and diclofenac in water samples by *in-situ* suspended aggregate micro-extraction (*i*SAME) method
2. To optimize FESI-Sweep-MSS on-line preconcentration capillary electrophoresis for the identification of pharmaceuticals in water samples.

1.8 Scope of study

This study will focus on the pharmaceuticals that are present in a specific sampling site in Johor River water. Hospital runoff give high impact in water problem when the discharges of pharmaceuticals are not monitored. Thus, monitoring level of pharmaceuticals is important as to ensure pharmaceuticals are correctly managed to ensure the safety of water bodies.

The extraction steps involve filtration of water sample using 0.45 μm cellulose membrane. *i*SAME was chosen to extract the pharmaceuticals by using different polarities of solvents and extraction times.

For analytical analysis, CE was used for screening step and optimizing step for the detection of these pharmaceuticals. This analysis was conducted in the Chemical Analysis Laboratory, UIRL, UTM Skudai, Johor Bahru.

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APPENDIX B

List of Publication from this Study

1. Nur Hidayati Jamil, Jafariah Jaafar, Ambavaram Vijaya Bhaskar Reddy, Zaiton Abdul Majid, Azmi Aris, Zulkifli Yusof. (2019). Characterization of selected pharmaceutical micro-pollutants in river water using *in-situ* suspended aggregate micro-extraction and field enhancement sample injection-capillary electrophoresis. *Indian Journal of Forensic Medicine & Toxicology*. Vol 13. No 4.
2. A. Vijaya Bhaskar Reddy, Zulkifli Yusop, Jafariah Jaafar, Nur Hidayati Jamil (2018). Developement and Validation of Capillary Electrophoresis Method for Simultaneous Determination of Six Pharmaceuticals in Different Food Samples Combining On-line and Off-line Sample Enrichment Techniques. *Food Analytical Methods*. Vol 11, No 2, ISSN 1936-9751.