LIQUID AND SOLID PHASE MICROEXTRACTION METHODS FOR THE ANALYSIS OF ORGANIC ENVIRONMENTAL POLLUTANTS

LOH SAW HONG

A thesis submitted in fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

MAY 2013
ACKNOWLEDGEMENTS

First and foremost, I would like to give my sincere thankfulness to my main supervisor, Prof. Dr. Mohd Marsin Sanagi, for his guidance and priceless support, scientific excitement, inspiration and patient throughout this research. I would like to thank my co-supervisors, Prof. Dr. Wan Aini Wan Ibrahim and Prof. Dr. Mohamed Noor Hasan and visiting Prof. Dr. Hassan Y. Aboul Enein for giving me suggestions and critical comments during SepSTec group meeting as well as reviewing my draft thesis and manuscripts.

I would like to thank my family members for their moral support. Though far away from my family, but I was blessed to have their full support and spiritual strength to complete my research and thesis writing. I sincerely appreciate the companion and entertainment from my helpful and humorous lab-mates, especially when I was upset. Not forgetting the science officers and lab assistants in Department of Chemistry, Faculty of Science, UTM, I would like to give my complete gratitude towards them for their helps.

Special thanks to UMT and MOHE for the study leave and financial sponsorship given to me for three years. Finally yet importantly, I would like to express my gratitude to Assoc. Prof. Dr. Marinah Arrifin for her prompt advice and suggestion during my first semester study in UTM.
ABSTRACT

This work involves the investigation of new approaches and applications in miniaturized sample preparation techniques based on liquid phase and solid phase microextractions. A two-phase hollow fiber liquid phase microextraction (HF-LPME) method combined with gas chromatography-mass spectrometry was developed for the determination of selected polycyclic aromatic hydrocarbons (PAHs) in fresh milk. Under optimized conditions, low detection limits (LODs) were obtained ranging from 0.07-1.4 µg L⁻¹ with relative recoveries of 85-110% which were higher than those obtained by conventional solvent extraction for the volatile PAHs. Agarose film liquid phase microextraction (AF-LPME) was developed for the extraction and preconcentration of PAHs in environmental water samples. Agarose, a green polymer, has been manipulated for different microextraction approaches. Agarose film was used as an interface between donor and acceptor phases which allowed for selective extraction of the analytes under optimum conditions. Under the optimum extraction conditions, the method showed good linearity in the range of 0.1–200 µg L⁻¹, low limits of detection (0.01-0.04 µg L⁻¹) and satisfactory relative recoveries (92.9-104.7%). AF-LPME device proved to be low-cost and thus recycle of the film was not required to eliminate the analytes carry-over between runs. A new microextraction technique termed agarose gel liquid phase microextraction (AG-LPME) was developed for the extraction of PAHs in water. Solvent-impregnated agarose gel disc used in AG-LPME was prepared by slicing gelled agarose and exchanging the solvent from water to ethanol and then to 1-octanol that functioned as the extractant and impregnation solvent. The solvent impregnated AG-LPME was found to be comparable with HF-LPME in terms of extraction efficiencies without solvent dissolution problems observed. The method offered high enrichment factors in the range of 89-177 and trace level LODs in the range of 9-14 ng L⁻¹. This technique combines extraction and preconcentration approaches using an environmentally-compatible solvent holder that fulfils the green chemistry concept. Due to the hydrophilic property of agarose, the selectivity of AG-LPME was evaluated on hydrophilic triazine herbicides. The AG-LPME showed significantly higher extraction efficiencies as compared to HF-LPME. The method offered superior enrichment factors in the range of 115-300 and trace LODs in the range of 0.02-0.04 µg L⁻¹. Multi-walled carbon nanotube-impregnated agarose film microextraction (MWCNT-AFME) combined with micro high performance liquid chromatography–ultraviolet detection has also been developed. The method utilized MWCNTs immobilized in agarose film which served as the adsorbent holder. The technique achieved trace LODs in the range of 0.1-50 ng L⁻¹ for selected PAHs. The new MWCNT-AFME method was successfully applied to the analysis of spiked green tea beverage samples with good relative recoveries. The results supported the feasibility of agarose to serve as adsorbent holder in solid phase microextraction, thus saving the cost of chemical and waste disposal.
ABSTRAK

Kerja ini melibatkan kajian pendekatan dan aplikasi baru dalam teknik mini penyediaan sampel berdasarkan pengekstrakan mikro fasa ceceair dan pepejal. Kaedah pengekstrakan mikro fasa ceceair membran gentian berongga (HF-LPME) jenis dua fasa digabung dengan kromatografi gas-spektrometri jisim dibangunkan untuk menentukan hidrokarbon aromatik polisiklik (PAHs) dalam susu segar. Pada keadaan optimum, had pengesanan (LOD) rendah (0.07-1.4 µg L⁻¹) diperoleh dengan perolehan balik relatif 85-110% yang lebih tinggi daripada pengekstrakan pelarut konvensional untuk PAHs merup. Pengekstrakan mikro fasa ceceair filem agarosa (AF-LPME) dibangunkan untuk mengekstrak dan pra-memekatkan PAHs dalam air persekitaran. Agarosa, sejenis polimer hijau, telah dimanipulasikan untuk pendekatan pengekstrakan mikro berlaiman. Filem agarosa digunakan sebagai antaramuka di antara fasa penderma dan penerima untuk membolehkan pengekstrakan selektif analit pada keadaan optimum. Pada keadaan pengekstrakan optimum, kaedah ini menunjukkan kelinearan baik dalam julat 0.1-200 µg L⁻¹, LOD rendah (0.01-0.04 µg L⁻¹) dan perolehan balik relatif memuaskan (92.9-104.7%). Oleh kerana peralatan AF-LPME berkos rendah, penggunaan semula atau pengitaran semula filem agarosa tidak diperlukan bagi menghindari pencemaran analit antara larian. Satu teknik pengekstrakan mikro baru dinamakan pengekstrakan mikro fasa ceceair gel agarosa (AG-LPME) dibangunkan bagi menentukan PAHs dalam air. Cakera gel agarosa terkandung pelarut yang diguna dalam AG-LPME disediakan dengan memotong agarosa yang telah membentuk gel dan menukar pelarut daripada air kepada 1-oktanol yang berfungsi sebagai pelarut pengekstrak dan impregnasi. Kaedah ini didapati setanding dengan HF-LPME dari segi keberkesanan pengekstrakan tanpa masalah kehilangan pelarut. Kaedah ini menawarkan faktor pengkayaan yang tinggi (89-177) dan LOD aras surih dalam julat 9-14 ng L⁻¹. Teknik ini menggabungkan pengekstrakan dan pra-pemekatan menggunakan pemegang pelarut yang serasi dengan persekitaran dan bersesuaian konsep kimia hijau. Disebabkan sifat agarosa yang hidrofilik, kepelitian AG-LPME seterusnya dinilai menggunakan racun rumpai triazina hidrofilik. AG-LPME menunjukkan keberkesanan pengekstrakan yang ketara lebih tinggi berbandingkan HF-LPME. Kaedah ini menawarkan faktor pengkayaan unggul (115-300) dan LOD aras surih dalam julat 0.02-0.04 µg L⁻¹. Pengekstrakan mikro karbon tiub-nano berbilang dinding yang terkandung dalam filem agarosa (MWCNT-AFME) digabung dengan kromatografi ceceair prestasi tinggi mikro-pengesanan ulra lembayung telah dibangunkan. Kaedah ini menggunakan karbon tiub-nano berbilang dinding yang tidak bergerak dalam filem agarosa untuk berfungsi sebagai pemegang bahan penjerap. Teknik ini mencapai LOD aras surih dalam julat 0.1-50 ng L⁻¹ bagi PAHs terpilih. Kaedah MWCNT-AFME baru ini telah berjaya diaplikasikan dalam analisis minuman teh hijau yang dipukukan dengan perolehan semula relatif yang baik. Keputusan kajian ini menyokong kebolehan agarosa untuk berfungsi sebagai pemegang bahan penjerap pengekstrakan mikro fasa pepejal dan menjimakatkan kos bahan kimia dan pelupusan sisa.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>ii</td>
<td></td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
<td></td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iv</td>
<td></td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
<td></td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vi</td>
<td></td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xii</td>
<td></td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
<td></td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xviii</td>
<td></td>
</tr>
<tr>
<td>LIST OF SYMBOLS</td>
<td>xxi</td>
<td></td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xxiii</td>
<td></td>
</tr>
</tbody>
</table>

## 1 INTRODUCTION

1.1 Background of the Problem  
1.2 Statement of Problem  
1.3 Research Objectives  
1.4 Scope of the Study  
1.5 Significance of Study

## 2 LITERATURE REVIEW

2.1 The Occurrence and Physical Properties of Polycyclic Aromatic Hydrocarbons  
2.2 Toxicity of PAHs  
2.2.1 PAHs-Induced Carcinogenesis  
2.2.2 Effects of PAHs on the Immune System  
2.3 PAHs Residues and Legislation
2.4 Sample Preparation Techniques for PAHs Residues in Water and Food
   2.4.1 Extraction and Clean-up of PAHs in Water 10
   2.4.2 Extraction and Clean-up of PAHs in Food 10
      2.4.2.1 Fatty Food 11
      2.4.2.2 Non Fatty Food 12
2.5 Alternative Green Microextraction Techniques 13
   2.5.1 Supercritical Fluid Extraction 14
   2.5.2 Subcritical or Superheated Water Extraction 14
   2.5.3 Solid Phase Microextraction 16
      2.5.3.1 Basic Principles of SPME 18
   2.5.4 Stir Bar Sorptive Extraction (SBSE) 18
   2.5.5 Microextraction in Packed Syringe (MEPS) 20
   2.5.6 Membrane Protected Micro Solid Phase Extraction 21
      2.5.7 Liquid Phase Microextraction 22
         2.5.7.1 Basic Principles of LPME 25
      2.5.8 Dispersive Liquid-liquid Microextraction 27
2.6 Summary of Past Reports on PAHs Analysis 28
2.7 Triazine Herbicides 34
2.8 Physical and Chemical Properties of Agarose 37
2.9 Application of Agarose 39

3 DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN FRESH MILK BY HOLLOW FIBER LIQUID PHASE MICROEXTRACTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY
   3.1 Introduction 40
   3.2 Experimental 42
      3.2.1 Chemicals and Reagents 42
      3.2.2 Materials 42
      3.2.3 Chromatographic Conditions 43
3.2.4 Hollow Fiber Liquid Phase Microextraction (HF-LPME) 43
3.2.5 Saponification prior to Solvent Extraction 44
3.2.6 Validation of Analytical Method 45
3.3 Results and Discussion 45
3.4 Conclusions 51

4 AGAROSE FILM LIQUID PHASE MICROEXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN WATER 52
4.1 Introduction 52
4.2 Experimental 54
  4.2.1 Chemicals and Reagents 54
  4.2.2 Materials 54
  4.2.3 Chromatographic Conditions 55
  4.2.4 Preparation of Agarose Film 55
  4.2.5 Agarose Film Liquid Phase Microextraction (AF-LPME) 56
  4.2.6 Validation of Analytical Method 57
  4.2.7 Sample Analysis 57
4.3 Results and Discussion 58
  4.3.1 Optimization of AF-LPME 58
    4.3.1.1 Stirring Speed 58
    4.3.1.2 Extraction Time 59
    4.3.1.3 Salting Out Effect 60
    4.3.1.4 Agarose Concentration 61
    4.3.1.5 Acceptor Phase Volume 63
  4.3.2 Validation of AF-LPME 64
  4.3.3 Application of AF-LPME on Environmental Water Samples 66
  4.3.4 Comparison with Other Reported Methods 67
4.4 Conclusions 70
SOLVENT-IMPREGNATED AGAROSE GEL LIQUID PHASE MICROEXTRACTION FOR THE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS AND TRIAZINE HERBICIDES

5.1 Introduction
5.2 Experimental
  5.2.1 Chemicals and Reagents
  5.2.2 Materials
  5.2.3 Chromatographic Conditions
  5.2.4 Preparation of Solvent Impregnated Agarose Gel Disc
  5.2.5 Solvent-Impregnated Agarose Gel Liquid Phase Microextraction (AG-LPME)
  5.2.6 Polypropylene Hollow Fiber Liquid Phase Microextraction (HF-LPME)
  5.2.7 Agarose Film Liquid Phase Microextraction (AF-LPME)
  5.2.8 Sample Analysis
5.3 Results and Discussion
  5.3.1 Optimization of Solvent-Impregnated AG-LPME for the Analysis of PAHs
    5.3.1.1 Stirring Speed
    5.3.1.2 Extraction Time
    5.3.1.3 Agarose Concentration
    5.3.1.4 Length of Agarose Gel Disc
  5.3.2 Optimization of HF-LPME for the Analysis of PAHs
  5.3.3 Comparison of Extraction Efficiencies of PAHs Among AG-LPME, AF-LPME and HF-LPME.
  5.3.4 Validation of AG-LPME for the Analysis of PAHs
5.3.5 Application of AG-LPME on Drinking Water Samples for the Analysis of PAHs
5.3.6 Optimization of Solvent-Impregnated AG-LPME for the Analysis of Triazine Herbicides
  5.3.6.1 Stirring Speed
  5.3.6.2 Extraction Time
  5.3.6.3 Sample pH
  5.3.6.4 Salting Out Effect
5.3.7 Optimization of HF-LPME for the Analysis of Triazine Herbicides
5.3.8 Comparison of Extraction Efficiencies of Triazine Herbicides between AG-LPME and HF-LPME
5.3.9 Validation of AG-LPME for the Analysis of Triazine Herbicides
5.4 Conclusions

6 MULTI-WALLED CARBON NANOTUBE-IMPREGNATED AGAROSE FILM MICROEXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN GREEN TEA BEVERAGE
6.1 Introduction
6.2 Experimental
  6.2.1 Chemicals and Materials
  6.2.2 Chromatographic Conditions
  6.2.3 Preparation of Multi-Walled Carbon Nanotube-Impregnated Agarose Film (MWCNT-AF)
  6.2.4 Multi-Walled Carbon Nanotube-Impregnated Agarose Film Microextraction (MWCNT-AFME)
6.2.5 Validation of Analytical Method 106
6.2.6 Sample Analysis 106
6.3 Results and Discussion 107
6.3.1 Optimization of MWCNT-AFME 107
   6.3.1.1 Conditioning Solvent 107
   6.3.1.2 Sample Volume, Concentration of MWCNTs and Number of Films 107
   6.3.1.3 Desorption Time and Desorption Solvent 111
   6.3.1.4 Stirring Speed and Extraction Time 113
6.3.2 Validation of MWCNT-AFME 115
6.3.3 Application of MWCNT-AFME on Green Tea Beverage Samples 117
   6.3.4 Comparison with Other Reported Methods 117
6.4 Conclusions 119

7 CONCLUSIONS AND FUTURE DIRECTIONS 120
7.1 Conclusions 120
7.2 Future Directions 122

REFERENCES 124
Appendices A - D 143-146
### LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Physical properties of several PAHs.</td>
<td>6</td>
</tr>
<tr>
<td>2.2</td>
<td>Published methods for the extraction and determination of PAHs from milk samples.</td>
<td>29</td>
</tr>
<tr>
<td>2.3</td>
<td>Published methods for the extraction and determination of PAHs from aqueous samples.</td>
<td>31</td>
</tr>
<tr>
<td>2.4</td>
<td>Published methods for the extraction and determination of PAHs from tea samples.</td>
<td>34</td>
</tr>
<tr>
<td>2.5</td>
<td>Published methods for the extraction and determination of triazine herbicides from aqueous samples.</td>
<td>36</td>
</tr>
<tr>
<td>3.1</td>
<td>Validation data of HF-LPME of PAHs from milk.</td>
<td>49</td>
</tr>
<tr>
<td>3.2</td>
<td>Fat contents and slopes of calibration plots of different milk samples.</td>
<td>49</td>
</tr>
<tr>
<td>3.3</td>
<td>PAHs residues in commercial fresh milk products (n=3).</td>
<td>50</td>
</tr>
<tr>
<td>3.4</td>
<td>Relative recovery study.</td>
<td>51</td>
</tr>
<tr>
<td>4.1</td>
<td>Characterization of agarose film.</td>
<td>63</td>
</tr>
<tr>
<td>4.2</td>
<td>Validation data of AF-LPME of PAHs from spiked river water sample, n=3</td>
<td>65</td>
</tr>
<tr>
<td>4.3</td>
<td>Relative recovery study of AF-LPME on river water</td>
<td>66</td>
</tr>
<tr>
<td>4.4</td>
<td>Application of AF-LPME on environmental water samples.</td>
<td>67</td>
</tr>
<tr>
<td>4.5</td>
<td>Comparison of the AF-LPME with other published methods for the extraction and determination of PAHs from water samples.</td>
<td>69</td>
</tr>
<tr>
<td>5.1</td>
<td>Validation data of AG-LPME of PAHs from spiked drinking water samples (n=3).</td>
<td>89</td>
</tr>
</tbody>
</table>
5.2 Relative recovery studies of AG-LPME using PAHs spiked drinking water.

5.3 Application of AG-LPME on drinking water samples for the analysis of PAHs (n=3).

5.4 Validation data of AG-LPME of triazine herbicides using spiked drinking water samples (n=3).

5.5 Relative recovery studies of AG-LPME using triazine herbicides spiked drinking and river water samples (n=3).

6.1 Validation data of MWCNT-AFME of PAHs from green tea beverage samples (n=3).

6.2 Relative recovery studies of MWCNT-AFME using spiked green tea beverage samples (n=3).

6.3 Application of MWCNT-AFME on green tea beverage samples (n=3).

6.4 Comparison of the MWCNT-AFME with other published methods for the extraction and determination of PAHs from tea samples.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Schematic of SPME procedures.</td>
<td>16</td>
</tr>
<tr>
<td>2.2</td>
<td>Stir bar coated with PDMS.</td>
<td>19</td>
</tr>
<tr>
<td>2.3</td>
<td>Schematic of SPMTE (See et al., 2010).</td>
<td>20</td>
</tr>
<tr>
<td>2.4</td>
<td>Design of membrane protected micro solid phase extraction.</td>
<td>21</td>
</tr>
<tr>
<td>2.5</td>
<td>Configuration A and B in LPME (Psillakis and Kalogerakis, 2003).</td>
<td>23</td>
</tr>
<tr>
<td>2.6</td>
<td>Two-phase and three-phase sampling modes LPME (Psillakis and Kalogerakis, 2003).</td>
<td>24</td>
</tr>
<tr>
<td>2.7</td>
<td>Schematic of cone-shaped LPME (Sanagi et al., 2007).</td>
<td>25</td>
</tr>
<tr>
<td>2.8</td>
<td>Schematic of DLLME procedures.</td>
<td>27</td>
</tr>
<tr>
<td>2.9</td>
<td>Chemical structure of agarose.</td>
<td>37</td>
</tr>
<tr>
<td>2.10</td>
<td>(a) Basic repeat units of agarose; (b) Schematic of gelling process of agarose (Zhou et al., 2006a).</td>
<td>39</td>
</tr>
<tr>
<td>3.1</td>
<td>Schematic of HF-LPME.</td>
<td>44</td>
</tr>
<tr>
<td>3.2</td>
<td>Effect of stirring speed on HF-LPME of PAHs in milk.</td>
<td>46</td>
</tr>
<tr>
<td>3.3</td>
<td>Effect of salting out on HF-LPME of PAHs in milk.</td>
<td>47</td>
</tr>
<tr>
<td>3.4</td>
<td>Effect of extraction time on HF-LPME of PAHs in milk.</td>
<td>48</td>
</tr>
<tr>
<td>4.1</td>
<td>(a) Agarose film and (b) glass tube that is used in agarose film liquid phase microextraction.</td>
<td>56</td>
</tr>
<tr>
<td>4.2</td>
<td>Schematic of AF-LPME system.</td>
<td>57</td>
</tr>
<tr>
<td>4.3</td>
<td>Effect of stirring speed on AF-LPME of PAHs from water sample.</td>
<td>59</td>
</tr>
<tr>
<td>4.4</td>
<td>Effect of extraction time on AF-LPME of PAHs from water sample.</td>
<td>60</td>
</tr>
</tbody>
</table>
4.5 Effect of salting out on AF-LPME of PAHs from water sample.

4.6 Effect of agarose concentration on AF-LPME of PAHs from water samples.

4.7 FESEM image of the 0.8% agarose film.

4.8 Effect of acceptor phase volume on AF-LPME of PAHs from water sample.

4.9 GC-MS analysis of four polycyclic aromatic hydrocarbons spiked at 100 µg L⁻¹ of river water on Agilent HP5 MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness).

5.1 (a) Agarose gel disc; (b) Agarose gel discs dipped in 70% ethanol solution.

5.2 Schematic of AG-LPME system.

5.3 Effect of stirring speed on AG-LPME of PAHs from water sample.

5.4 Effect of extraction time on AG-LPME of PAHs from water sample.

5.5 Effect of agarose concentration on AG-LPME of PAHs from water sample.

5.6 Effect of agarose disc length on AG-LPME of PAHs from water sample.

5.7 Effect of stirring speed on HF-LPME of PAHs from water sample.

5.8 Effect of extraction time on HF-LPME of PAHs from water sample.

5.9 Comparison of extraction efficiencies among AG-LPME, AF-LPME and HF-LPME. Error bars followed by same letter without string showed no significant difference according to Anova, Tukey test p>0.05.
5.10 GC-MS total ion chromatogram (TIC) of four PAHs obtained after (a) AG-LPME and (b) HF-LPME spiked at 40 µg L⁻¹ of deionized water. Conditions: helium constant flowrate of 1 mL min⁻¹, oven temperature profile was programmed at 150°C for 3 min, and then increased to 250°C at 10°C min⁻¹ using Agilent HP5 MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness).

5.11 Effect of stirring speed on AG-LPME of triazines from water sample.

5.12 Effect of extraction time on AG-LPME of triazines from water sample.

5.13 Effect of sample pH on AG-LPME of triazines from water sample.

5.14 Effect of salting out on AG-LPME of triazines from water sample.

5.15 Effect of stirring speed on HF-LPME of triazines from water sample.

5.16 Effect of extraction time on HF-LPME of triazines from water sample.

5.17 Comparison of extraction efficiencies between AG-LPME and HF-LPME. Error bars followed by same letter without string showed no significant difference according to t-test, p>0.05.

5.18 GC-MS total ion chromatogram (TIC) of three triazine herbicides obtained after (a) AG-LPME and (b) HF-LPME spiked at 4 µg L⁻¹ of deionized water. Conditions: helium constant flowrate of 1 mL min⁻¹, oven temperature profile was programmed at 170°C, and then increased to 206°C at 3°C min⁻¹ using Agilent HP5 MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness).

5.19 GC-MS TIC of (a) drinking and (b) river water samples that were free from triazines and used for relative recovery studies.
6.1 Schematic drawing of MWCNT-AFME system. 106
6.2 Effect of sample volume on MWCNT-AFME of PAHs from water sample. 108
6.3 Effect of concentration of MWCNTs on MWCNT-AFME of PAHs from water sample. The surface area of the 0.1, 0.3 and 0.6% of MWCNTs impregnated within the agarose film were 21.4, 37.6 and 52.0 m$^2$ g$^{-1}$, respectively. 109
6.4 FESEM image of the (a) 0.3% MWCNT-AF and (b) AF; the pictures on the right top corner of each image were the actual image of the circular films. 110
6.5 Effect of pieces of MWCNTs-AF on MWCNT-AFME of PAHs from water sample. 111
6.6 Effect of desorption solvent on MWCNT-AFME of PAHs from water sample. 112
6.7 Effect of stirring speed on MWCNT-AFME of PAHs from water sample. 113
6.8 Effect of extraction time on MWCNT-AFME of PAHs from water sample. 114
6.9 µ-HPLC-UV analysis of three PAHs spiked at 0.008 µg L$^{-1}$ for both PHE and FLA and 5 µg L$^{-1}$ for BaP of green tea beverage on Agilent ZORBAX Eclipse Plus C$_{18}$ column (2.1 x 100 mm, 3.5 µm). µ-HPLC conditions: isocratic mobile phase ACN-water (80:20) (v/v), column temperature at 25°C, flowrate at 0.2 mL min$^{-1}$, injection volume of 2 µL and detection wavelength at 254 nm. 116
LIST OF ABBREVIATIONS

ACE - Acetone
ACT - Acetonitrile
AF - Agarose film
AF-LPME - Agarose film liquid phase microextraction
AG - Agarose gel
AG-LPME - Agarose gel liquid phase microextraction
AOAC - Association of Analytical Communities
ATR - Atrazine
BaP - Benzo[a]pyrene
BET - Brunauer Emmett Teller
CYN - Cyanazine
DAD - Diode array detector
DCM - Dichloromethane
DLLME - Dispersive liquid-liquid microextraction
DNA - Deoxyribonucleic acid
EF - Enrichment factor
EME - Electromembrane extraction
EtOH - Ethanol
EU - European Union
FD - Fluorescence detector
FESEM - Field emission scanning electron microscope
FLA - Fluoranthene
FLU - Fluorene
GC - Gas chromatography
HF - Hollow fiber
HF-LPME - Hollow fiber liquid phase microextraction
HPLC - High performance liquid chromatography
HS - Headspace
I.D. - Internal diameter
IL - Ionic liquid
IPA - Isopropyl alcohol
LLE - Liquid-liquid extraction
LOD - Limit of detection
LOQ - Limit of quantification
LPME - Liquid phase microextraction
LVI - Large volume injection
MASE - Membrane assisted solvent extraction
ME - Microextraction
MEPS - Microextraction in packed syringe
MRL - Maximum residue level
MS - Mass spectrometry
MWCNT-AF - Multi-walled carbon nanotube-impregnated agarose film
MWCNT-AF ME - Multi-walled carbon nanotube-impregnated agarose film microextraction
MWCNTs - Multi-walled carbon nanotubes
NaCl - Sodium chloride
PAHs - Polycyclic aromatic hydrocarbons
PCBs - Polychlorinated biphenyls
PDA - Photodiode array
PDMS - Polydimethylsiloxane
PE - Polyethylene
PHE - Phenanthrene
PP - Polypropylene
PTV - Programmed temperature vaporization
PYR - Pyrene
RSD - Relative standard deviation
RTP - Room temperature phosphorimetry
SBSE - Stir bar sorptive extraction
SDME - Single drop microextraction
SE - Solvent extraction
SEC - Secbumeton
SFE - Subcritical fluid extraction
SFO - solidification of floating organic drop
SIM - Selected ion monitoring
SIR - Solvent impregnated resin
SPE - Solid phase extraction
SPME - Solid phase microextraction
SPMTE - Solid phase membrane tip extraction
SWE - Subcritical or superheated water extraction
THF - Tetrahydrofuran
USEPA - United States Environmental Protection Agency
UV - Ultraviolet
WHO - World Health Organization
µHPLC - Micro high performance liquid chromatography
µ-SPE - Micro solid phase extraction
# LIST OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$pK_a$</td>
<td>Acid dissociation constant</td>
</tr>
<tr>
<td>$n$</td>
<td>Amount of analyte extracted by the coating</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>$R^2$</td>
<td>Coefficient of determination</td>
</tr>
<tr>
<td>$C_w$</td>
<td>Concentration of analyte in the aqueous sample solution</td>
</tr>
<tr>
<td>$C_0$</td>
<td>Concentration of analyte in the organic extraction solvent</td>
</tr>
<tr>
<td>$r$</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>$^\circ C$</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>$eV$</td>
<td>Electronvolt</td>
</tr>
<tr>
<td>$K$</td>
<td>Equilibrium distribution coefficient</td>
</tr>
<tr>
<td>$K_{fs}$</td>
<td>Fiber coating/sample matrix distribution constant</td>
</tr>
<tr>
<td>$V_f$</td>
<td>Fiber coating volume</td>
</tr>
<tr>
<td>$g$</td>
<td>Gram</td>
</tr>
<tr>
<td>$g \text{ mol}^{-1}$</td>
<td>Gram per mole</td>
</tr>
<tr>
<td>$h$</td>
<td>Hour</td>
</tr>
<tr>
<td>$C_0^w$</td>
<td>Initial amount of the analyte</td>
</tr>
<tr>
<td>$C_0$</td>
<td>Initial concentration of the analyte in the sample</td>
</tr>
<tr>
<td>$kV$</td>
<td>Kilovolts</td>
</tr>
<tr>
<td>$L$</td>
<td>Liter</td>
</tr>
<tr>
<td>$m$</td>
<td>Meter</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Micro</td>
</tr>
<tr>
<td>$\mu g$</td>
<td>Microgram</td>
</tr>
<tr>
<td>$\mu g \text{ g}^{-1}$</td>
<td>Microgram per gram</td>
</tr>
<tr>
<td>$\mu g \text{ kg}^{-1}$</td>
<td>Microgram per kilogram</td>
</tr>
<tr>
<td>$\mu g \text{ L}^{-1}$</td>
<td>Microgram per liter</td>
</tr>
<tr>
<td>$\mu m$</td>
<td>Micrometer</td>
</tr>
<tr>
<td>$mg$</td>
<td>Milligram</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>mg L(^{-1})</td>
<td>Milligram per liter</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>ng L(^{-1})</td>
<td>Nanogram per liter</td>
</tr>
<tr>
<td>K(_{ow})</td>
<td>1-octanol/water partitioning coefficients</td>
</tr>
<tr>
<td>ppb</td>
<td>Part per billion</td>
</tr>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>(p)</td>
<td>Probability</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>(V_s)</td>
<td>Sample volume</td>
</tr>
<tr>
<td>s</td>
<td>Seconds</td>
</tr>
<tr>
<td>(V_w)</td>
<td>Volume of aqueous sample solution</td>
</tr>
<tr>
<td>(V_o)</td>
<td>Volume of organic extraction solvent</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per volume</td>
</tr>
</tbody>
</table>
## LIST OF APPENDICES

<table>
<thead>
<tr>
<th>APPENDIX</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>List of Publications from this Study</td>
<td>143</td>
</tr>
<tr>
<td>B</td>
<td>List of Awards from this Study</td>
<td>144</td>
</tr>
<tr>
<td>C</td>
<td>List of Presentations Related to this Study</td>
<td>145</td>
</tr>
<tr>
<td>D</td>
<td>List of Patents Related to this Study</td>
<td>146</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Background of the Problem

Green Chemistry, a phrase first coined by the United States Environmental Protection Agency (USEPA) in the early 1990s as ‘to promote innovative chemical technologies that reduce or eliminate the use or generation of hazardous substances in the design, manufacture and use of chemical products’ (Environmental Protection Agency, 2008). Chemists are concerned about the future of the environment and are worried about what will happen if we continue to suffocate the earth with all the hazardous substance in chemical process. Therefore, it is important to design or develop greener chemical processes and to utilize sustainable development practices in order to eliminate environmental degradation and pollution.

Conventional extraction methods such as liquid-liquid extraction or solvent extraction based on organic solvents are generating hazardous wastes to the environment, and this has contradicted the concept of green chemistry which emphasises on chemical process or technology that improves the environment and quality of life (Sanghi and Srivastava, 2003).

In order to meet green chemistry requirements, eco-friendly extraction and innovations for a cleaner analysis is urged. Of this, solid phase microextraction (SPME) and liquid phase microextraction (LPME) have emerged as the latest green analysis to replace solid phase extraction (SPE) and liquid-liquid extraction (LLE).
The techniques are categorized as ‘prevention’ under the twelve principles of green chemistry proposed by Anastas and Warner in 1991 (Anastas and Warner, 2000).

1.2 Statement of Problem

Although the green techniques such as SPME and LPME have been increasingly developed and modified, the application of the techniques are still confined to the research institutes and universities. This is mainly because most of the green techniques were in-house validated. The food manufacturers and private industries’ chemists are still employing the conventional technique such as LLE that was declared as standard reference method by some of the associations who established the maximum residue levels of food and environmental pollutants. Therefore, the publication of the green techniques should be made available to those associations and the public.

As fresh milk is used as one of the substitutes for breast milk during infancy, a rapid, selective, sensitive method is required to ensure the fresh milk is safe to be consumed. The monitoring of environmental pollutants residue levels is important to establish new maximum permitted levels. Existing method that uses conventional solvent extraction and fat saponification to extract environmental pollutants from milk consumes large amount of organic solvent and the procedures are tedious. Therefore, an environmentally green extraction method shall be developed to replace the existing solvent extraction method in order to reduce organic solvent usage and disposal costs. The liquid phase microextraction (LPME) promotes the reduction of hazardous substances in chemical processes for sample extraction and analysis.

In order to resolve the solvent dissolution problem that occurs during LPME, hollow fiber liquid phase microextraction (HF-LPME) has been developed to replace the core single drop microextraction (SDME). The extraction solvent or acceptor phase is well protected under impregnated hollow fiber made of polypropylene. However, solvent depletion problems are still frequently reported even though
hollow fiber is used. In addition, although polypropylene is a recycled thermoplastic, the economic and environmental benefit of recycling is always an argument issue of the environmentalists. Therefore, an alternative material to protect acceptor phase in LPME is an urge to solve those problems.

1.3 Research Objectives

The objectives of this research are:

a. To develop and apply hollow fiber liquid microextraction (HF-LPME) method combined with gas chromatography–mass spectrometry (GC-MS) for the analysis of polycyclic aromatic hydrocarbons (PAHs) in fresh milk;

b. To develop an innovative LPME system using biodegradable material termed agarose film (AF) and solvent impregnated agarose gel (AG) LPME coupled with GC-MS for the analysis of PAHs in water samples;

c. To evaluate the selectivity of hydrophilic agarose towards hydrophilic triazine herbicides using solvent impregnated AG-LPME;

d. To develop an innovative multi-walled carbon nanotube-impregnated agarose film microextraction (MWCNT-AFME) combined with micro high performance liquid chromatography (μHPLC)-ultraviolet (UV) detection for the analysis of PAHs in green tea beverage samples.

1.4 Scope of the Study

Liquid phase microextraction techniques were thoroughly studied where the extraction performances of commercially available hollow fiber and innovations on biodegradable agarose were evaluated. Hollow fiber liquid phase microextraction (HF-LPME) technique was studied for the first time to extract lipophilic PAHs from fresh milk samples. Several important extraction parameters were optimized and sample pretreatment steps such as pH adjustment and ultrasonification of sample solutions have been omitted. Agarose which is generally used in biotechnology
analysis as a medium of deoxyribonucleic acid (DNA) electrophoresis separation were prepared into film and solvent impregnated gel forms to serve as barriers or membranes of LPME to replace the hollow fiber. Several extraction parameters were explored and the newly developed technique was compared with LPME using commercially available HF for PAHs analysis as the reference method. The newly developed solvent–impregnated agarose gel LPME was thoroughly studied on its selectivity towards analytes which are more hydrophilic. Triazine herbicides were selected as model compounds. Several extraction parameters were comprehensively optimized and the optimum conditions were applied for the analysis of environmental and drinking water samples. The results were compared with those obtained by HF-LPME. An innovative agarose-immobilized sorbent film microextraction was studied, where multi-walled carbon nanotube (MWCNT)-impregnated agarose film was synthesized and applied as adsorbent film in solid phase microextraction (SPME). Several extraction parameters were thoroughly investigated and optimized for the analysis PAHs in green tea beverage samples.

1.5 Significance of Study

Modern life depends on the petrochemical and chemical related industries - most drugs, food, transportation, office equipments, paints, analysis and plastics are derived from oil and chemicals. It is difficult to imagine what will happen when petroleum-based starting materials become more expensive when the population growth is accelerated against world oil production. At the commencement of the new century, a shift in emphasis in green chemistry is apparent with the desire to develop more environmentally friendly routes. From a green chemistry viewpoint, the uses of green solvent and material have many advantages. The idea of “green” solvents or materials expresses the goal to minimize the environmental impact and reduce the waste produced and chemical related impact on human health resulting from the use of solvents in chemical process and analysis. For this reason, finding environmentally green-solvent and material have become a top priority of the chemists. Therefore, the use of less organic solvent and biodegradable material as extraction tools have formed the main thrust of a movement.
REFERENCES


