MOLECULARLY IMPRINTED POLYMER SOLID PHASE EXTRACTION FOR THE ANALYSIS OF ORGANOPHOSPHORUS PESTICIDES IN FOOD SAMPLES

SYAIRAH BINTI SALLEH

UNIVERSITI TEKNOLOGI MALAYSIA

MOLECULARLY IMPRINTED POLYMER SOLID PHASE EXTRACTION FOR THE ANALYSIS OF ORGANOPHOSPHORUS PESTICIDES IN FOOD SAMPLES

SYAIRAH BINTI SALLEH

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Science (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

APRIL 2012

To my beloved mama and ayah,
Hajah Fatimah Binti Haji Kasmani and Haji Salleh Bin Haji Megat Abdullah,
my family members and those who are close to me.

ACKNOWLEDGEMENT

Alhamdulillah, Alhamdulillah, Alhamdulillah. First and foremost, all praise to Allah, the Almighty God for with His grace that has given me the strength, patience, and the time to complete this work.

I am deeply thankful to my supervisor Prof. Dr. Mohd Marsin Sanagi for his patience, supervision, helpful suggestions, advice, encouragement and thoughtful guidance towards the completion of this thesis.

I would like to extend my greatest gratitude to my co-supervisors, Dr Ahmedy Abu Naim and Prof. Dr. Wan Aini Wan Ibrahim, and thanks to Dr Dadan Hermawan and all group members in the Separation Science and Technology Group (SepSTech) for their support, and to everyone who has encouraged me and assisted me over the years.

I also gratefully acknowledge the Ministry of Higher Education (MOHE) and Ministry of Science, Technology and Innovation (MOSTI) Malaysia for financial support through "Bajet Mini" and National Science Fellowship (NSF). Thanks also to all staff of the Department of Chemistry and Ibnu Sina Institute for Fundamental Science Studies, UTM for their full friendliness and assistance in handling instruments and also supplying relevant literature and information.

Also thanks to my friends who have provided assistance in towards completion of this thesis. Last but not least, a very warm thank you to my beloved family especially my parents for their support, love and most precious care.

ABSTRACT

A new material based on molecularly imprinted polymers (MIP) was prepared as solid phase extraction (SPE) sorbent for sample enrichment in the analysis of organophosphorus pesticides (OPPs). The polymer with binding sites situated on the surface shows many advantages including high selectivity and high recoveries towards the target analytes. The MIP was synthesized by non-covalent imprinting technique using quinalphos as the template molecule, methacrylic acid (MAA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as crosslinker and acetonitrile as porogen solvent. The physical characteristics of the MIP were characterized using fourier transform infrared spectrometry (FTIR), CHN analyzer, field emission scanning electron microscopy (FE-SEM), nitrogen adsorption and UV-VIS spectrometry. The three OPPs (diazinon, quinalphos and chlorpyrifos) were selected as target analytes as they are widely used in agriculture sector. Various parameters and conditions affecting the extraction efficiency of the imprinted polymers were evaluated to optimize the selective preconcentration of OPPs compounds from aqueous samples. By using 100 mg of sorbent, it was found that the optimum MIP-SPE conditions were: 10 mL loading sample volume, 30% of acetonitrile in water as washing solvent, 5% acetic acid in methanol as elution solvent, and 6 mL elution solvent volume. The extracts were analyzed by high performance liquid chromatography coupled with ultraviolet-visible detection at 200 nm. The accuracy and selectivity of the MIP-SPE method developed were verified with non-imprinted polymer solid phase extraction (NIP-SPE) and commercial C_{18}-SPE for comparison. The MIP-SPE showed superior extraction efficiency towards the three selected OPPs compared to the NIP-SPE and commercial C_{18}-SPE. The limits of detection (LOD) of OPPs for MIP-SPE ranged from 0.83 μg L^{-1}-2.8 μg L^{-1} with the percentage recovery of greater than 91%. Meanwhile, the LODs of OPPs for NIP-SPE and C_{18}-SPE were in the range of 3.77 μg L^{-1}-6.14 μg L^{-1} and 2.87 μg L^{-1}-3.12 μg L^{-1}, respectively and percentage recoveries of greater than 62% and 88%, respectively. The developed method was successfully applied to the analysis of OPPs in two selected fruit samples namely, grapes and green apples. The analysis of OPPs in samples using the developed MIP-SPE showed good results with recoveries of 89.74%-99.70% and RSDs of less than 3%.

ABSTRAK

Bahan berkepilihan baru berdasarkan polimer cap molekul (MIP) telah disediakan sebagai bahan penjerap pengesktrakan fasa pepejal (SPE) dalam pemekatan sampel analisis racun organofosforus (OPPs). Polimer dengan lokasi ikatan pada permukaannya menunjukkan banyak kelebihan termasuk keterpilihan tinggi dan peratus pengembalian tinggi bagi analit sasaran. MIP telah disediakan dengan teknik pencetakan bukan kovalen menggunakan kuinalfos sebagai molekul templat, asid metakrilik (MAA) sebagai monomer berfungsi, etilena glikol dimetakrilat (EGDMA) sebagai bahan silang dan asetonitril sebagai pelarut porogen. Sifat fizik MIP telah diuji dengan spektroskopi inframerah transformasi fourier (FTIR), analisis CHN, mikroskop imbasan elektron-pancaran medan (FE-SEM), penjerapan nitrogen dan UV-VIS spestroskopi. Tiga OPPs (diazinon, kuinalfos dan klorpirifos) dipilih sebagai analit sasaran kerana ia digunakan secara meluas dalam sektor pertanian. Pelbagai parameter dan keadaan yang mempengaruhi kecekapan pengekstrakan polimer cap itu telah dinilai bagi mengoptimumkan pra-pemekatan berkepilihan bagi sebatian OPPs dalam sampel akueus. Dengan menggunakan bahan penjerap 100 mg, didapati keadaan optimum MIP-SPE adalah: pemuatan isipadu sampel 10 mL; campuran 30% asetonitril dalam air sebagai pelarut pembilas, campuran 5% asetik asid dalam pelarut metanol sebagai pelarut pengelusian analit dan isipadu pelarut pengelusian analit 6 mL. Ekstrak telah dianalisis menggunakan kromatografi cecair prestasi tinggi berganding dengan pengesanan ultralembayung-nampak pada 200 nm. Ketepatan dan kepilihan kaedah MIP-SPE yang dibangunkan telah diuji dengan menggunakan pengesktrakan fasa pepejal polimer bukan cap (NIP-SPE) dan komersial C_{18}-SPE sebagai perbandingan. Kaedah MIP-SPE menunjukkan kecekapan pengekstrakan yang lebih baik terhadap ketiga-tiga OPPs terpilih berbanding dengan pengekstrakan menggunakan NIP-SPE dan C_{18}-SPE komersial. Had pengesanan (LOD) OPPs untuk MIP-SPE adalah dalam julat 0.83 μg L^{-1}-2.8 μg L^{-1} dengan peratus pengembalian melebihi 91%. Sementara itu, kaedah NIP-SPE dan C_{18}-SPE komersial memberi had pengesanan (LOD) dalam julat 3.77 μg L^{-1}-6.14 μg L^{-1} dan 2.87 μg L^{-1}-3.12 μg L^{-1} masing-masing dan peratusan pengembalian melebihi 62% dan 88%, masing-masing. Kaedah yang dibangunkan telah berjaya diaplikasikan bagi analisis OPPs dalam buah-buahan terpilih, iaitu, anggur dan epal hijau. Analisis OPPs dalam sampel buah-buahan menggunakan kaedah MIP-SPE memberikan keputusan yang baik dengan pengembalian 74%-99.70% dan sisihan piawai relative (RSD) kurang daripada 3%.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

MIP	-	Molecularly imprinted polymer
SPE	-	Solid phase extraction
NIP	-	Non imprinted polymer
C_{18}	-	Octadecyl carbon chain
MIP-SPE	-	Molecularly imprinted polymer solid phase extraction
LLE	-	Liquid-liquid extraction
OPPs	-	Organophosphorus pesticides
OCP	-	Organochlorine pesticides
EU	-	European Union
EEC	-	Estimated environmental concentration
EC	-	European Council
HPLC-UV	-	High performance liquid chromatography-Ultraviolet
RP-HPLC	-	Reverse phase-High performance liquid chromatography
MAA	-	Methacrylic acid
EGDMA	-	Ethylene glycol dimethacrylate
AIBN	-	Azobisisobutyronitrile
PTFE	-	Teflon
FTIR	-	Fourier transform infrared
FE-SEM	-	Field emission-scanning electron microscopy
UV-VIS	-	Ultraviolet visible
EPA	-	Environmental Protection Agency
DOA	-	Department of Agriculture
MOA	-	Ministry of Agriculture
PS-DVB	-	Polystyrene-divinylbenzene
RSD	-	Relative standard deviation
CE	-	Capillary electrophoresis
LOD	-	Limit of detection

LOQ	-	Limit of quantification
Log Kow	-	Octanol/water partition coefficient
Is	-	Internal standard
LD	-	Lethal Dose
LC	-	Lethal Concentration
GC	-	Gas chromatography
min	-	minutes
mL	-	mililiter
mmol	-	milimol
ppb	-	part per billion
ppm	-	part per million
mV	-	miliVolt
mL min^{-1}	-	milliliter per minute
µL	-	microliter
µm	-	micrometer

CHAPTER 1

INTRODUCTION

1.1 Research Background

A molecularly imprinted polymer (MIP) is a polymer that is formed in the presence of a molecule (called template) that is extracted afterwards, thus leaving complementary cavities behind (Glad *et al.,* 2000). MIPs are tailor-made materials with high selectivity for target molecules. Many MIPs have been prepared and utilized mainly as affinity chromatography media. The resulting imprinted polymers are stable and robust. In addition, it is important to point out that synthesis of MIP is also relatively cheap and easy as compared with other selective materials such as immunosorbent (IS), thus making MIP a clear alternative to the use of natural receptors (Pichon, 2007).

One of the most exciting applications of MIPs is as sorbent for solid-phase extraction (SPE). In SPE, the sample is passed through a cartridge or a packed column filled with a solid sorbent where the analytes are absorbed and then eluted with an organic solvent. This procedure presents several advantages: particularly it is less time consuming than liquid-liquid extraction (LLE) procedure, it decreases the use of toxic solvents and offers the possibility of automation (Andersson, 2000; Pichon and Hugon, 2008). Despite their attractive features, the classical SPE sorbents such as C_{18}, ion-exchange and size-exclusion phases are lacking in selectivity towards target analytes. In order to overcome this drawback, the use of MIPs in SPE (MIP-SPE) has been developed (Jiang *et al.,* 2008; Caro *et al.,* 2006; Han *et al.,* 2005; Fang *et al.,* 2005; Boer *et al.,* 2002). MIP-SPE allows not only the

analyte to be pre-concentrated but also the other compounds present in the sample matrix to be removed.

Over the last 60 years, farmers and growers have been using pesticides for food production in order to meet the expectation of consumers, increasing production and quality in food production. Consequently, the consumers are exposed to pesticides usually in little quantities in a number of food groups such as vegetable, fruits and juices. Organophosphorous pesticides (OPPs) are one of the most common classes of pesticides involved in poisoning because of the inhibition of acetyl-cholinesterase (Sultafos, 2008). *Monitoring and analysis the* trace level of OPPs in food and environmental contamination are therefore essential for human health protection and environmental control. The maximum residue limits (MRLs) of pesticide residues in fruits and vegetables set by the European Communities depend on types of fruits or vegetables (*European Economic Community* (EEC), 1976). However, the regulation stated a default limit of 0.01 mg kg^{-1} for all pesticides combinations in food which has no set MRLs (European Communities, 2005). In addition, the European Union (EU) has established a *maximum allowable concentration* of a single compound for drinking water quality of 0.1 μg L^{-1} for individual pesticides and 0.5 μg L^{-1} for the total concentration of all pesticides (*European Economic Community* (EEC), 1980). The Malaysian Food Regulation 1985 (pesticides residue, Part VII) has stated that the maximum residue limits for all pesticides were as recommended by the Codex Alimentarius Commission (Joint FOA/WHO Food Standards) and 0.01 miligram per kilogram for any pesticide residues that are not listed in Codex Alimentarius (Food Regulations 1985; Codex Alimentarius Commission 2010).

1.2 Problem Statement

The trace levels determination of organic contaminants in complex matrices requires sample pretreatment and enrichment processes because of high levels of interferences. Solid-phase extraction (SPE) is routinely used for the extraction of compounds from liquid or solid matrices. However, most of the classical SPE

sorbents such as C_{18}, ion-exchange and size-exclusion phases do not show high selectivity for specific analytes. Recently, MIPs have attracted considerable attention to be used as SPE sorbents to get high selectivity and high recoveries towards the target analytes. In addition, MIPs offer cleanup and preconcentration of target analytes prior to determination.

Organophosphorus pesticides (OPPs) are important compounds to analyze as over the last several years OPPs contamination of drinking water and agricultural products has become a major concern and the number of OPPs is steadily increasing. Recently, molecularly imprinted solid-phase extraction method was used for the analysis of OPPs in river water sample, with extraction recoveries ranging from 77.5% to 99.1% (Zhu *et al.,* 2005). However, their work was limited to only the relatively polar OPPs (monocrotophos, mevinphos, phosphamidon, omethoate). Hence, in this work, a new MIP was synthesized based on O,O-diethyl O-2-quinoxalinyl phosphorothioate (quinalphos) as a template for use in MIP-SPE sample enrichment of selected non polar OPPs (quinalphos, diazinon and chlorpyrifos) in fruit samples prior to HPLC-UV analysis. To the best of our knowledge, no report has been published on MIP-SPE of these OPPs.

1.3 Research Objectives

The research objectives are as follows:

1.3.1 To prepare a molecularly imprinted polymer (MIP) using an organophosphorus pesticide (OPP) as the template.

1.3.2 To characterize structure and surface morphology of the MIP.

1.3.3 To apply the MIP as solid-phase extraction (SPE) sorbent for the analysis of organophosphorus pesticides prior to HPLC.

1.3.4 To compare the selectivity and extraction efficiency between the newly prepared MIP sorbent and commercial C18-SPE sorbent in the determination of organophosphorus pesticides in samples.

1.4 Scope of Study

In this study a new selective material based on molecularly imprinted polymers (MIP) was prepared for sample preparation in the analysis of organophosphorus pesticides (OPPs). Three OPPs were considered in this study, namely quinalphos, diazinon, and chlorpyrifos. The physical and chemical characteristics of the MIP were characterized using fourier transform infrared spectrometry (FTIR), CHN analyzer, field emission scanning electron microscopy (FE-SEM), nitrogen adsorption and UV-VIS spectrometry. The MIP was applied as SPE sorbent (MIP-SPE) and in the analysis of OPPs. The results of the analysis were compared with non imprinted polymer as solid phase extraction sorbent (NIP-SPE) and commercial C_{18}-SPE. The identification and quantification of OPPs was carried out using high pressure liquid chromatography with ultraviolet detection (HPLC-UV) at 200 nm.

Optimization of MIP synthesis parameters were carried out to evaluate the affinity and imprinting effects. The parameters chosen in this study were types of crosslinker and porogen solvents. The optimized parameters of MIP-SPE, NIP-SPE and commercial C_{18}-SPE were washing solvents, loading volumes, elution solvent types and elution volumes. The extraction efficiency of the MIP-SPE was compared with NIP-SPE and commercial C18-SPE under identical extraction conditions. The MIP-SPE method was validated in terms of linearity, limit of detection and limit of quantification. The developed method was applied to fruit samples, namely grape and green apple.

REFERENCES

Ahmed, F. E. (2001). Analyses of Pesticides and Their Metabolites in Food and Drinks. *Trends Anal. Chem.* 20 (11), 649-653.

Albericio, F. and Puche, J. T. (2008). *The Power of Functional Resins in Organic Synthesis*. (pp. 28-30). Heppenheim : John Wiley & Sons, Inc.

Andersson, L. I (2000). Molecular Imprinting: Developments and Applications in the Analytical Chemistry Field. *J. Chromatogr. A.* 745, 3.

Baggiani, C., Trotta, F., Giraudi, G., Giovannolia, C., and Vanni, A. (1999). A Molecularly Imprinted Polymer for the Pesticide Bentazone. *Anal. Commun*. 36, 263.

Baggiani, C., Baravalle, P., Giraudi, G. and Tozzi, C. (2007). Molecularly Imprinted Solid-Phase Extraction Method for the High-Performance Liquid Chromatographic Analysis of Fungicide Pyrimethanil in Wine. *J. Chromatogr. A.* 1141, 158.

Bereczki, A., Tolokan, A., Horvai, G., Horvath, V., Lanza, F., Hall, A. J. and Sellergren, B. (2001). Determination of Phenytoin in Plasma by Molecularly Imprinted Solid-Phase Extraction. *J. Chromatogr. A.* 930, 31-38.

Biotage Company, http://www.biotage.com/DynPage.aspx?id=93583 (accessed on February 2011).

Boer, T. D., Mol, R., de Zeeuw, R. A., de Jong, G.J., Sherrington, D. C., Cormack, P. A. G. and Ensing, K. (2002). Spherical Molecularly Imprinted Polymer Particles: a Promising Tool for Molecular Recognition in Capillary Electrokinetic Separations. *Electrophoresis.* 23, 1296.

Bruggemann, O. (2001). Catalytically Active Polymers Obtained by Molecular Imprinting and Their Application in Chemical Reaction Engineering. *Biomol Eng*. 18. 4.

Brune, B. J., Koehler, J. A., Smith, P. J. and Payne, G. F. (1999). Correlation between Adsorption and Small Molecule Hydrogen Bonding. *Langmuir*. 15. 3989.

Buchmeiser, M. R. (2001). New Synthetic Ways for the Preparation of High Performance Liquid Chromatograhy. *J. Chromatogr*. 918, 233-266.

Cacho. C., Turiel. E., Esteban, M. A., Conde, P. C. and Camara. C. (2003). Cleanup of Triazines in Vegetable Extracts by Molecularly-Imprinted Solid-Phase Extraction using a Propazine-Imprinted Polymer. *Anal Bioanal Chem*. 376, 491–496.

Cacho, C., Turiel, E. and Conde, C. P. (2009). Molecularly Imprinted Polymers: An Analytical Tool for the Determination of Benzimidazole Compounds in Water Samples. *Talanta*. 78, 1029-1031.

Caro, E., Masque, E., Marce, R. M., Borrull, F., Cormack, P. A. G. and Sherrington, D. C. (2002). Non-Covalent and Semi-Covalent Molecularly Imprinted Polymers for Selective Online Solid-Phase Extraction of 4-Nitrophenol from Water Samples. *J. Chromatogr. A.* 963 (1-2), 169.

Caro, E., Marce, R. M., Borrull, F., Cormarck, P. A. G. and Sherrington, D.C. (2004). A New Molecularly Imprinted Polymer for the Selective Extraction of Naproxen from Urine Samples by Solid-Phase Extraction. *J. Chromatogr*. 813, 137–143.

Caro, E., Marce, R. M., Borrull, F., Cormarck, P. A. G. and Sherrington, D. C. (2006). Application of Molecularly Imprinted Polymers to Solid-Phase Extraction of Compounds from Environmental and Biological Samples. *Trend Anal. Chem*. 25, 143.

Chapuis, F., Pichon, V., *Lanza,* F., Sellergren, B. and Hennion, M. C. (*2004)*. Retention Mechanism of Analytes in the Solid-Phase Extraction Process Using Molecularly Imprinted Polymers. *J. Chromatogr. B.* 804, 97.

Chimuka, L., Cukrowska, E. and Johnson, J. A. (2004). Why Liquid Membrane Extraction is an Attractive in Sample Preparation. *Pure Appl. Chem.* 76. 707-722.

Cormack, P. A. G. and Elorza, A. Z. (2004). Molecularly Imprinted Polymers: Synthesis and Characterization. *J. Chromatogr. B.* 804, 174–178.

Codex Alimentarius Commission 2010. Website for Pesticide Residues in Food, Available online: http://www.codexalimentarius.net/pestres/data/index.html (accessed on March 2012)

Department of Agriculture, DOA and Ministry of Agriculture, MOA (Crop Statistical Data), http://www.doa.gov.my/web/guest/data perangkaan tanaman (accessed on March 2012)

Dong, X. C., Wang, N., Wang, S. L., Zhang, X. W. and Fan, Z. J. (2004). Synthesis and Application of Molecularly Imprinted Polymer on Selective Solid-Phase Extraction for the Determination of Monosulfuron Residue in Soil. *J Chromatogr. A.* **1057**, 13–19.

Edson, J. H. (1955). *Factor Influencing the Demand for Pesticides in Tropical Agricultural*. Advances in Chemistry Series 13, American Chemical Society, Washington D. C., 9-13.

European Economic Community (EEC) of 23 November 1976 relating to the fixing of maximum levels for pesticide residues in and on fruit and vegetables**,** http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX: 31976L0895:EN:HTML (accessed on 19 October 2011).

European Economic Community (EEC), 1980. Offic. *J. Euro. Commun.* No. L 229,30.08 (1980) 11.

Europian Communities (EC) Regulation 396/2005 on Pesticide Regulation, http://www.food.gov.uk/safereating/chemsafe/pesticides/pesticidesmainqa/ecregulation (accessed on 19 October 2011).

Farrington, K., Magner, E. and Regan, F. (2006). Predicting the Performance of Molecularly Imprinted Polymers: Selective Extraction of Caffeine by Molecularly Imprinted Solid Phase Extraction. *Anal. Chim. Acta*. 566, 60–8.

Fang, G. Z., Tan, J. and Yan, X. P. (2005). An Ion-Imprinted Functionalized Silica Gel Sorbent Prepared by A Surface Imprinting Technique Combined with A Sol-Gel Process for Selective Solid-Phase Extraction of Cadmium (II). *Anal. Chem.* 77, 1734.

Feng, Q. Z., Zhao, L. X., Yana, W., Lana, J. M. and Zheng, Z. X. (2009). Molecularly Imprinted Solid-Phase Extraction Combined with High Performance Liquid Chromatography for Analysis of Phenolic Compounds from Environmental Water Samples. *J. Hazard. Mater*. 170, 1247-1255.

Fest, C. and Schmidt, K. J. (1982). *The Chemistry of Organophosphorus Pesticides.* New York: Lewis Publishers.

Flores, A., Cunliffe, D., Whitcombe, M. J. and Vulfson, E. N. (2000). Imprinted Polymers Prepared by Aqueous Suspension Polymerization. *J. Appl. Polym. Sci.* 77 (8), 1841-1850.

Food Regulations 1985. Pesticide Residue. Available online: http://fsis2. moh. gov. mt/Fosimv2/HOM/frmHOMFARSecDetail.aspx?FAS_ID=36&FAC_ID=21&FAD_ID=238 (accessed on March 2012)

Fontanals, N., Marce. R. M. and Borrull. F. (2005). New Hydrophilic Materials for Solid Phase Extraction. *Trends Anal. Chem*. 24, 394-405.

Fritz, J. S. (1999). *Analytical Solid-Phase Extraction*. New York: Wiley-VCH, 264.

Gallardo, E., Barroso, M., Margalho, C., Cruz, A., Vieira, D. N. and Rivadulla, L. M. (2006). Determination of Quinalphos in Blood and Urine by Direct Solid-

Phase Microextraction Combined with Gas Chromatography-Mass Spectrometry. *J. Chromatogr. B.* 832, 162-168.

Glad, M., Norlow, O., Sellergren, B., Siegbahn, N. and Mosbach, K. (2000*).* Use of Silane Monomers for Molecular Imprinting and Enzyme Entrapment in Polysiloxane-Coated Porous Silica*. J. Chromatogr. A.* 347, 11.

Goldman, L. R. (1997). *Raw and Processed Food Schedule for Pesticides Tolerance Reassessment*. United State: Environmental Protection Agency (EPA).

Gonzalez, M. E. L. and Arribas, L. V. P. (2000). Chemically Modified Polymeric Sorbents for Sample Preconcentration. *J. Chromatogr*. 902, 3-16.

Han, D. M., Fang, G. Z. and Yan, X. P. (2005). Preparation and Evaluation of A Molecularly Imprinted Sol–Gel Material for On-Line Solid-Phase Extraction Coupled With High Performance Liquid Chromatography for the Determination of Trace Pentachlorophenol in Water Samples. *J. Chromatogr. A.* 1100, 131.

Hamilton, D. and Crossley, S. (2004). *Pesticides Residue in Food and Drinking Water: Human Exposure and Risks.* (pp. 28-38). Chichester: John Wiley & Son.

Harvey, S. D. (2005). Molecularly Imprinted Polymers for Selective Analysis of Chemical Warfare Surrogate and Nuclear Signature Compounds in Complex Matrices. *J. Sep. Sci.* 28, 1221-1230.

He, C. Y., Long, Y. Y., Pan, J. L., Li, K. A. and Liu, F. (2007). Application of Molecularly Imprinted Polymers to Solid-Phase Extraction of Analytes from Real Samples. *J. Biochem. Biophys. Methods*. 70, 133-137.

Hennion, M. C. and Pichon, V. (2003). Immuno-Based Sample Preparation for Trace Analysis. *J. Chromatogr. A*. 1000, 29.

Henry, M. (2000). *SPE Technologies: Principles and Application*. N. J. K. Simpson, ed., New York; Marcel Dekker, 125-182.

http://extoxnet.orst.edu/pips/diazinon.html (accessed on January 2011).

http://www.termiteweb.com/chlorpyrifos/ (accessed on January 2011).

http://www.chemexper.com/chemicals/supplier/cas/13593-03-8.html (accessed on December 2010).

Hunt, J. W., Anderson, B. S., Phillips, B. M., Nicely, P. N., Tjeerdema, R. S., Puckett, H. M., Stephenson, M., Worcester, K. and Vlaming, D. V. (2003). Ambient Toxicity Due to Chlorpyrifos and Diazinon in a Central California Coastal Watershed. *Environ. Monit. Assess.* 82(1), 83-112.

Hylton, K. and Mitra, S. (2007). Automated Analysis via Membrane Extraction. *J. Chromatogr. A.* 1152, 199-214.

Irani, M. (2003). Pesticides Residue in Vegetables in and around Delhi. *Environ. Monitor. Assess*. 86, 265-271.

Jiang, X., Zhao, C., Jiang, N., Zhang, H. and Liu, M. (2008). Selective Solid-Phase Extraction Using Molecular Imprinted Polymer for the Analysis of Diethylstilbestrol. *Food Chem.* 108, 1061.

Jiang, T., Zhao, L., Chua, B., Feng, Q., Yan, W. and Lin, J. M. (2009). Molecularly Imprinted Solid-Phase Extraction for the Selective Determination of 17β-Estradiol in Fishery Samples with High Performance Liquid Chromatography. *Talanta.* 78, 445.

Ju, W. P., Jun, Y., De, S. Q., Yun, G., Lan, Z. X. and Bao, C. J. (2007). Rapid Removal of Template from Molecularly Imprinted Polymers by Accelerated Solvent Extraction. *Chin. J. Anal. Chem*. 35, 486.

Kempe, M. and Mosbach, K. (1995). Molecular Imprinting Used for Chiral Separations. *J. Chromatogr. A.* 694, 3.

Kim, H. and Spivak, D. A. (2003). New Insight Into Modeling Non-Covalently Imprinted Polymers. *J. Am. Chem. Soc.* 125, 11269-11275.

Komiyama, M., Takeuchi, T., Mukawa, T. and Asanuma, H. (2003). *Molecular Imprinting-From Fundamentals to Applications.* Weinheim: Wiley-VCH. 17.

Masquee, N., Marcee, R. M. and Borrull, F. (2001). Molecularly Imprinted Polymers: New Tailor-Made Materials for Selective Solid-Phase Extraction. *Trends Anal. Chem*. 20, 480.

Matsui, J., Okada, M., Tsuruoka, M. and Takeuchi, T. (1997). Solid-phase Extraction of a Triazine Herbicide Using a Molecularly Imprinted Synthetic Receptor. *Anal. Commun.* 34, 85.

Mayes, A. G. and Mosbach, K. (1997). Molecularly Imprinted Polymers: Useful Materials for Analytical Chemistry. *Trends Anal. Chem.* 16, 321-332.

Michailof, C., Manesiotis, P. and Panayiotou, C. (2008). Synthesis of Caffeic Acid and p-Hydroxybenzoic Molecularly Imprinted Polymers and Their Application for the Selective Extraction of Polyphenol from Olive Mill Waste Waters. *J. Chromatogr. A.* 1182, 25.

Ministry of Agriculture, MOA (List of Registered Pesticides), http://www.doa.gov.my/web/guest/senarai racun perosak berdaftar (accessed on March 2012).

Molinelli, A., Weiss, R. and Mizaikoff, B. (2002). Advanced Solid Phase Extraction using Molecularly Imprinted Polymers for The Determination of Quercetin in Red Wine. *J. Agric. Food Chem.* 50. 1804-1808.

Molinelli, A. (2004). *Molecularly Imprinted Polymer: Towards a Rational Understanding of Biomimics Materials*. Ph.D. Thesis. Georgia Institute of Technology, School of Chemistry and Biochemistry. p 32.

Muldon, M. T. and Stanker, L. H. (1995). Polymer Synthesis and Characterization of a Imprinted Sorbent Assay for Atrazine. *J. Agric. Food Chem*. 43, 1424.

Nakamura, M., Ono, M., Nakajima, T., Ito, Y., Aketo, T. and Haginaka, J. (2005). Uniformly Sized Molecularly Imprinted Polymer for Atropine and Its Application to the Determination of Atropine and Scopolamine in

Pharmaceutical Preparations Containing *Scopolia* Extract. *J. Pharm. Biomed. Anal.* 37, 231.

Pandey, S. and Singh, D. K. (2004). Total Bacterial And Fungal Population After Chlorpyrifos and Quinalphos Treatments in Groundnut (Arachis hypogaea L). *Chemosphere*. 55 (2), 197-205.

Puoci, F., Garreffa, C., Iemma. F., Muzzalupo, R., Spizzirri. U. G. and Picci, N. (2005). Molecularly Imprinted Solid Phase Extraction for Detection of Sudan I in Food Matrices. *Food Chem*. 93, 349–353.

Pichon, V., Bouzige, M., Miege, C. and Hennion, M. C. (1999). Immunosorbents: Natural Molecular Recognition Materials for Sample Preparation of Complex Environmental Matrices. *Trends Anal. Chem.* 18, 219.

Pichon, V. (2007). Selective Sample Treatment using Molecular Imprinted Polymer. *J. Chromatogr. A.* 1152, 41-42

Pichon, V. and Hugon, F. C. (2008). Role of Molecularly Imprinted Polymer for Selective Determination of Environmental Pollutants - A Review. *J. Chim. Acta.* 622, 50.

Puranik, S. B., Pawar. V. R., Lalitha, N., Pai. P. N. S. and Rao, G. K. (2009). Residual Solvent Analysis in Hydrochloride Salts of Active Pharmaceutical Ingredients. *Pak. J. Pharm. Sci*. 22, 412.

Racke, K. D. (1992). Degradation of Organophosphorus Insecticides in Environmental Matrices. In: Chambers JE, Levi PE, eds. *Organophosphates: Chemistry, Fate, and Effects*. (pp. 47-78). San Diego, CA: Academic Press.

Reigart, J. R. and Roberts, J. R. (1999). *Recognition And Management Of Pesticide Poisonings.* (pp. 34, 94, 137, 196). Washington: EPA's Office of Pesticide Programs, U.S. Environmental Protection Publications.

Remcho, V. T. and Tan, Z. J. (1999). MIPs as Chromatographic Stationary Phases for Molecular Recognition. *Anal. Chem.* 71, 248A.

Sanagi, M. M. (1998). *Teknik Pemisahan Dalam Analisis Kimia*. Johor Bahru: UTM Press. p 51.

Sanagi, M. M., Sulaiman, A. and Wan Ibrahim, W. A. (2004). *Principles of Chemical Analysis*. Johor Bahru: UTM Press.

Sellergren, B. (1994). Direct Drug Determination by Selective Sample Enrichment on an Imprinted Polymer of Special Interest. *Anal. Chem*. **66**, 1578–1582.

Sellergren, B. (1997). Noncovalent Molecular Imprinting: Antibody-Like Molecular Recognition in Polymeric Network Materials. *Trends Anal. Chem*. 16, 310–320.

Sellergren, B. (1999). Polymer- and Template-Related Factors Influencing the Efficiency in Molecularly Imprinted Solid-Phase Extractions. *Trends Anal. Chem*. **18**, 164.

Sellergren B. (2001). Molecularly Imprinted Polymers-Man-Made Mimics of Antibodies and their Applications in Analytical Chemistry. *Anal. Chem.* 23, *113*−184.

Shi, Y., Zhang, J. H., Shi, D., Jiang, M., Zhu, Y. X., Mei, S. R., Zhou, Y. K., Dai, K. and Lu, B. (2006). Selective Solid-Phase Extraction of Cholesterol Using Molecularly Imprinted Polymers and Its Application In Different Biological Samples. *J. Pharm. Biomed. Anal.* 42, 555.

Song, X., Li, J., Wang, J. and Chen, L. (2009). Quercetin Molecularly Imprinted Polymers: Preparation, Recognition Characteristics and Properties as Sorbent for Solid Phase Extraction. *Talanta.* 80, 698.

Stuart, B. H. (2002). *Polymer Analysis*. Chichester: John Wiley & Sons, *Ltd*. pp.121-170.

Sultafos, L. G. (2008). Mammalian Toxicology of Organophosphorus Pesticides. *J. Toxicol. Environ. Health*. 43, 271–289.

Svec, F., Tennikova, T. B. and Deyl. Z. (2003). *Monolithic Materials: Preparation, Properties, and Applications.* (pp. 689). Netherland: Elsevier Publishing.

Temple, W. A. and Smith, N. A. (1989). *Organophosphorus Pesticides*. New Zealand:Inchem (www.inchem.org) (accessed on 10 December 2007).

U.S. Environmental Protection Energy - Types of Pesticides, http://www.epa.gov/ pesticides/about/types.htm. (Last updated on Friday, November 04, 2011), (accessed on March 2012).

United States Court of Appeals, California Table Grape Commission, updated on 31 March 2008, http://caselaw.findlaw.com/us-federal-cicuit/1593241.html, (accessed on March 2012).

United States Department of Agriculture (USDA) - Fresh Deciduous Fruit Annual, updated on 10/27/2011, http://www. globaltrade.net / f/ market-reserah/ pdg / south-Africa/Agriculture-Animal-Husbandadry-Hunting-Fishing-Deciduous-Fruit-Season-Dampened-by-Flood. html, (accesed on March 2012)

Viana, E., Redondo, M. J., Font, G. and Molto, J. C. (1996). Disk versus Columns in the Solid Phase Extraction of Pesticides from Water. *J. Chromatogr*. 733, 267-274.

Wulff, G. and Sarhan, A. (1972). Use of Polymers with Enzyme-Analogous Structures for the Resolution of Racemates. *Angew. Chem. Int. Ed. Engl*. 11, 341.

Wulff, G., Vietmeier, J. and Poll, H. G. (1987). Influence of the Nature of the Cross Linking Agent on the Performance of Imprinted Polymers in Racemic Resolution. *Makromol. Chem*. 188, 736.

Yan, H. and Row. K. H. (2006). Characteristic and Synthetic Approach of Moleculary Imprinted Polymer . *Int. J. Mol. Sci.* 7, 163.

Ye, L., Cormack, P. A. G. and Mosbach, K. (2001). Molecular Imprinting on Microgel Spheres. *Anal. Chim. Acta.* 435, 187–196.

Zander, A., Findlay, P., Renner, T. and Sellergen, B. (1998). *Analysis* of *Nicotine* and *Its* Oxidation Products in *Nicotine* Chewing Gum by a *Molecularly Imprinted Solid Phase Extraction. Anal. Chem*. 70, 3304.

Zhu, X., Yang, J., Su, Q., Cai, J. and Gao, Y. (2005). Selective Solid Phase Extraction using Molecularly Imprinted Polymer for Polar Organophosphorus Pesticides in Water and Soil Samples. *J. Chromatogr. A.* 1092, 161.

Zubrzycki, S. (1972). *Lectures in Probability Theory and Mathematical Statistics*. (pp. 239). New York: American Elsevier Publishing.

LIST OF PRESENTATIONS AND PUBLICATIONS

1. M. Marsin Sanagi, Syairah Salleh, Wan Aini Wan Ibrahim and Ahmedy Abu Naim, "Molecularly Imprinted Polymers For Solid Phase Extraction Of Organophosphorus Pesticides", poster presented at Regional Annual Fundamental Science Seminar 2010 (RAFSS 2010), Kuala Lumpur., 25 June 2010.

2. M. Marsin Sanagi, Syairah Salleh, Wan Aini Wan Ibrahim and Ahmedy Abu Naim, "Determination Of Organophosphorus Pesticides Using Molecularly Imprinted Polymer Solid Phase Extraction", paper presented at the The 23rd Regional Malaysian Symposium of Analytical Sciences (SKAM-23), Permai Inn, Kuala Terengganu. 4-6 October 2010.

3. M. Marsin Sanagi, Syairah Salleh, Wan Aini Wan Ibrahim, and Ahmedy Abu Naim., "Molecularly Imprinted Polymers For Solid Phase Extraction Of Organophosphorus Pesticides", *Journal of Fundamental Sciences*, Ibnu Sina UTM, Vol. 6, No. 1 (2010) 27-30; ISSN 1823-626X.

4. M. Marsin Sanagi, Syairah Salleh, Wan Aini Wan Ibrahim, and Ahmedy Abu Naim., "Molecularly Imprinted Polymers For Solid Phase Extraction Of Organophosphorus Pesticides", *The Malaysian Journal of Analytical Sciences*, Vol 15 No 2 (2011) 175-183; ISSN 1394-2506.