SELECTIVE SEPARATION OF PHENOLIC COMPOUND USING MOLECULAR IMPRINTING TECHNIQUE FOR SOLID PHASE EXTRACTION

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A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
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APRIL 2010

I declare that this thesis entitled "Selective Separation of Phenolic Compound using Molecular Imprinting Technique for Solid Phase Extraction" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Date : 30 April 2010

To my beloved family and fellow friends

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ABSTRACT

The objectives of this research are to study the performance of molecular imprinted polymer (MIP) in solid phase extraction (SPE) process and to determine the formulation for preparing of MIP particle and also to analyze the absorbance differences between polymer and silica. As we know, cocoa contained much higher levels of total phenolic compounds such as phenol. Phenolic compounds are widely distributed in the plant kingdom. This study is basically to adsorb phenol using molecular imprinting technique for solid phase extraction. They are two parameters used which are adsorbent amount and concentration of phenol solution to observe their effects of absorbance percentage and absorbance capacity. Furthermore, for adsorbent amount used in this study are 2g, 4g, 6g and 8g while the concentration of phenol used are 100mg/L, 200mg/L, 300mg/L, 400mg/L and 500mg/L. From the experimental result, it showed that the optimum adsorbent amount in this experiment is 5g while the optimum concentration of phenol is 300mg/L. Besides, the technique used in this study is molecular imprinting technique to prepare MIP particle for solid phase extraction. The successful preparation of molecularly imprinted polymers for solid phase extraction provides an innovative opportunity for the development of advanced adsorption phenolic compound in plant. The experimental results clearly showed that higher adsorbent amount and higher concentration of phenol solution gave higher absorbance. The experimental results clearly showed that higher adsorbent amount and higher concentration of phenol solution gave higher absorbance. A higher selectivity of target molecule proved when performing the extraction using polymer.

ABSTRAK

Kajian ini dijalankan bertujuan utk mengkaji kebolehan MIP untuk dijadikan sebagai penyerap dalam teknik SPE dan juga untuk mengkaji formula dalam menyediakan MIP untuk menghasilkan polimer serta untuk menganalisis perbezaan penyerapan oleh silika dan polimer yang telah dihasilkan. Sebagai mana yang kita sedia maklum, koko mengandungi kandungan fenol yang tinggi. Kandungan fenol juga sememangnya meluas dalam tumbuhan lain. Kajian ini secara amnya mengkaji penyerapan fenol dengan menggunakan teknik MIP untuk digunakan dalam teknik SPE. Terdapat dua parameter yang digunakan iaitu jumlah penyerap dan kepekatan larutan fenol untuk mengkaji kesannya kepada peratusan penyerapan. Bagi jumlah penyerap yang digunakan dalam kajian ini adalah 2g, 4g, 6g dan 8g manakala kepekatan larutan fenol pula adalah 100mg/L, 200mg/L, 300mg/L, 400mg/L dan 500mg/L. Kajian yang telah dijalankan menunjukkan bahawa jumlah optimum penyerap adalah 5g manakala kepekatan larutan fenol optimum pula adalah 300mg/L. Eksperimen ini juga jelas menunjukkan keputusan bahawa semakin tinggi jumlah penyerap dan kepekatan larutan fenol, semakin tinggi peratusan penyerapan serta molekul yang ditarget iaitu templat adalah lebih tinggi jika penyerap yang digunakan adalah polimer berbanding silika.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENTS	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	ix
	LIST OF FIGURES	x
	LIST OF ABBREVIATIONS	xii
	LIST OF APPENDICES	xiii
1	**INTRODUCTION**	1
	1.1 Background	1
	1.2 Problem Statement	3
	1.3 Objectives of Study	5
	1.4 Scopes of Study	5
2	**LITERATURE REVIEW**	6
	2.1 Phenolic compound	6
	2.2 Molecular Imprinting Technique (MIP)	8
	2.3 Solid Phase Extraction (SPE)	13
	2.3.1 Reversed Phase SPE	17
	2.4 Suspension Polymerization	22
	2.5 Hydrolysis	22

3	**METHODOLOGY**	25
	3.1 Materials	25
	3.2 Methodology	26
	3.2.1 Molecular Imprinting Technique (MIP) preparation	27
	3.2.2 Suspension Polymerization	28
	3.2.3 Solid Phase Extraction (SPE)	29
4	**RESULTS AND DISCUSSIONS**	31
	4.1 Effect of adsorbent amount for polymer.	31
	4.2 Effect of concentration for polymer.	32
	4.3 Effect of adsorbent amount on absorbance difference between polymer and silica.	33
	4.4 Effect of concentration on absorbance difference between polymer and silica.	33
	4.5 Difference of absorbance capacity between polymer and silica on the effect of adsorbent amount.	34
	4.6 Difference of absorbance capacity between polymer and silica on the effect of concentration.	35
	4.7 Differences of detection of phenol using FTIR.	36
5	**CONCLUSION AND RECOMMENDATION**	37
	5.1 Conclusion	37
	5.2 Recommendation	39
6	**REFERENCES**	40
	APPENDIX A	44
	APPENDIX B	52
	APPENDIX C	57
	APPENDIX D	59

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	SPE Phase Types	16
3.1	Polymer recipes	27
A.1	Effect of adsorbent amount for silica.	44
A.2	Effect of concentration for silica.	45
A.3	Effect of adsorbent amount for polymer (without template).	46
A.4	Effect of concentration for polymer (without template).	46
A.5	Effect of adsorbent amount polymer (with template).	46
A.6	Effect of concentration for polymer (with template).	47
A.7	Effect of adsorbent amount on absorbance capacity for polymer.	47
A.8	Effect of concentration on absorbance capacity for polymer.	48
A.9	Effect of adsorbent amount on absorbance capacity for silica.	49
A.10	Effect of concentration on absorbance capacity for silica.	50
C.1	Standard calibration curve.	57

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
1.1	Molecular structure of phenol.	3
2.1	Molecular Imprinting Technique (MIP)	13
2.2	Sep-Pak Vac 35cc. (silica C_{18} , 10g)	17
2.3	Solid Phase Extraction Steps	21
3.1	Flow of Methodology	26
3.2	Molecular imprinting polymer technique	28
3.3	Solid Phase Extraction	30
4.1	Effect of adsorbent amount for polymer	31
4.2	Effect of concentration for polymer	32
4.3	Effect of adsorbent amount on absorbance difference between polymer and silica.	33
4.4	Effect of concentration on absorbance difference between polymer and silica.	33
4.5	Difference of absorbance capacity between polymer and silica on the effect of adsorbent amount.	34
4.6	Difference of absorbance capacity between polymer and silica on the effect of concentration.	35
4.7	Differences of detection of phenol using FTIR	36
4.8	Phenol detected by FTIR.	36
A.1	Effect of adsorbent amount for silica.	44
A.2	Effect of concentration for silica.	45
A.3	Effect of adsorbent amount on absorbance capacity for polymer.	48
A.4	Effect of concentration on absorbance capacity for polymer.	49

A.5	Effect of adsorbent amount on absorbance capacity for silica.	50
A.6	Effect of concentration on absorbance capacity for silica.	51
C.1	Standard calibration curve.	58
D.1	Ground polymer with mortar and pestle.	59
D.2	Sieve tray at 200µm particle size.	59

LIST OF ABBREVATIONS

MIP	-	Molecular Imprinting Technique
SPE	-	Solid Phase Extraction
N_2	-	Nitrogen
MAA	-	Methacrylic acid
EDGMA	-	Ethylene glycol dimethacrylate
DMPAP	-	Dimethylaminophenol
UV	-	Ultraviolet
FTIR	-	Fourier Transform Infrared Spectroscopy
NaOH	-	Sodium hydroxide
IR	-	Infrared

LIST OF APPENDICES

APPENDICES	TITLE	PAGE
A	Experimental result	44
B	Calculation	52
C	Standard calibration curve	57
D	Picture of experiment	59

"I hereby declare that I have read this thesis and in my opinion this thesis has fulfilled the qualities and requirements for the award of Degree of Bachelor of Chemical Engineering (Biotechnology)"

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

One of the major sources of phenolic compound is cocoa. Cocoa beans come from the fruit of the cacao tree which grows in tropical rainforests in South America, Africa, and Malaysia. Ghana is one of the largest producers of high quality cocoa (Jonfiaessien, *et al.,* 2008). The official scientific name of the cocoa tree is *Theobroma Cacao*. "*Theobroma*" is Latin for "food of the gods". Cocoa (*Theobroma cacao L.)* is an important crop in the economics of several countries such as Ghana, Ivory Coast, Nigeria, Indonesia and Malaysia. Malaysia is the fifth largest producer of cocoa beans in the world. It is one of the main producers of cocoa-based products in the world and the biggest in Asia. However, Malaysian beans are sold at a lower price compared to the West African beans, due to some weaknesses in its quality (low cocoa aroma, astringent and bitter taste). One of the factors which could cause this could be a high amount of phenolic substances. A study done by Natsume *et al.* (2000) reported that phenolic content in cocoa liquor varied with the country of origin (A. Othman, *et al.,* 2005).

The words "cacao" and the more commonly used term "cocoa" both refer to the cacao bean, the seed of the *Theobroma Cacao* fruit. Strictly speaking, cocoa or cacao is a nut, the seed of a fruit, but is most commonly called cocoa beans, cocoa seeds, cocoa

nuts, chocolate seeds, or chocolate beans. Raw cocoa has the highest antioxidant value of all the natural foods in the world. The Oxygen Radical Absorbance Capacity (ORAC) score per 100 grams of unprocessed raw cacao is 28,000, compared to 18,500 for acai berries, 1,540 for strawberries, and only 1,260 for raw spinach. Cocoa beans contain 10,000 milligrams (10 grams) of phenolic compound per 100 grams (Jovanovic, 1994). Plant phenolic compounds are diverse in structure but are characterised by hydroxylated aromatic rings. They are categorised as secondary metabolites, and their function in plants is often poorly understood. Many plant phenolic compounds are polymerised into larger molecules such as the proanthocyanidins and lignins. Furthermore, phenolic acids may occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxyfatty acids, sterols, and glucosides (Sahelian *et al.,* 2006).

Black tea, green tea, red wine, and cocoa are also high in phenolic phytochemicals. Phenolic compounds in plant (tannins, lignins) serve as defenses against herbivores and pathogens. Cocoa contained much higher levels of total phenolics (611 mg of gallic acid equivalents) (W. Lee *et al.,* 2003). Phenolic compounds are widely distributed in the plant kingdom. Plant tissues may contain up to several grams per kilogram. External stimuli such as microbial infections, ultraviolet radiation, and chemical stressors induce their synthesis (Kahkonen *et al.,* 1999). MIP involves the synthesis of cross-linked polymers around a template molecule. Once the polymer has been formed the template is removed by washing, leaving an `imprint' of the analyte template. Ideally this gives a sorbent on which highly selective, reversible binding of the analyte can achieved. In recent years, solid-phase extraction (SPE) has become a very important technique for sample preparation because of its advantages over liquid-liquid extraction (J. Olsen *et al.,* 1998).

The analysis of phenolic compound has to be extracted selectively from the samples, resulting in the requirement of highly selective affinity phases for examples,

solid phase extraction (SPE) and membrane technique (Bruggemann *et al.,* 2003). This study would adsorb phenolic compound using molecular imprinting technique using solid phase extraction.

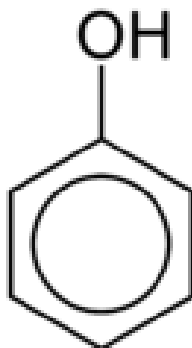

Figure 1.1 Molecular structure of phenol.

1.2 Problem Statement

Consumers all over the world are becoming more conscious of the nutritional value and safety of their food and its ingredients. At the same time, there is a preference for natural foods and food ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts (Swan *et al.,* 1979). Phenolic compound have become an intense focus of research interest because of their perceived beneficial effects for health including anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory, immune modulating, anti-microbial, vasodilatory and analgesic effects (Jonfiaessien *et al.,* 2008).

Phenolic compound in cacao used as ingredients in dietary supplements to prevent diseases such as cancer and coronary heart disease. Recent research has demonstrated that the antioxidants found in cacao beans are highly stable and easily

available to the human metabolism. This makes cacao is the most potent source of antioxidants and a source of the most usable antioxidants found in any natural food (Bruggemann *et al.,* 2003).

The use of news methodologies such as solid-phase extraction (SPE) has increased for the extraction of phenol compounds from liquid samples. Recently, highly selective extraction based on molecular imprinted polymers (MIP) has been developed. A molecular imprinted polymer (MIP) has become the method of choice in many laboratories for the analysis of complex samples.

1.3 Objective of Study

The objectives of this study are to study the performance of molecular imprinted polymer (MIP) in solid phase extraction (SPE) process, to determine the formulation for preparing of MIP particle and to analyze the absorbance differences between polymer and silica.

1.4 Scope of Study

In order to achieve the objectives, there are several scopes that we have to be focusing on which are the effect of adsorbent amount, concentration of phenol solution, and types of adsorbent.

CHAPTER 2

LITERATURE REVIEW

2.1 Phenolic compund

Food such as fruits, vegetables and grains are reported to contain a wide variety of antioxidant components, including phenolic compounds. These compounds are found to be well correlated with antioxidant potential. Phenolics or polyphenols have received considerable attention because of their physiological functions, including antioxidant, antimutagenic and antitumour activities (Azizah *et al.,* 1998).

Phenolic compounds seem to be universally distributed in plants. Thay have been the subject of a great number of chemical, biological, argriculture, and medical studies. Plant phenolic compounds are diverse in structure but are characterised by hydroxylated aromatic rings (e.g. flavan-3-ols). They are categorised as secondary metabolites, and their function in plants is often poorly understood. Many plant phenolic compounds are polymerised into larger molecules such as the proanthocyanidins (PA; condensed tannins) and lignins. Furthermore, phenolic acids may occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxyfatty acids, sterols, and glucosides. Phenol was isolated from coal tar in 1834. It

served as a bacteriocide in the late 19th century. Phenol extraction first served to purify carbohydrates. It was subsequently adapted to "purify" nucleic acids. It also separates glycoproteins from erythrocyte membrane non-glycoproteins (Karen, 1994). Phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl functional group (-OH) attached to an aromatic hydrocarbon group. The simplest of the class is phenol (C_6H_5OH). Some phenols are germicidal and are used in formulating disinfectants. Others possess estrogenic or endocrine disrupting activity (Sahelian *et al.,* 2006).

From Feingold definitions, phenol is a group of natural and synthetic compounds that are ingested or produced to vary degrees by the body or by microbes in the intestine contain benzene ring with one or more hydroxyl groups attached to it (Karen, 1994). Phenol can be purchased as a crystalline solid (with 0.1% hypophosphorous acid as antioxidant) or as liquified (88%) phenol. Distillation removes hypophosphorous acid from crystalline phenol or brown coloring (and oxidation products) from liquid phenol. Phenol gradually oxidizes producing a brown color. Phenol oxidizes by a free radical process (Karen, 1994).

Phenolic compounds are plant-based materials, phytochemicals. There may be 4,000 of these plant compounds, and only a few, such as Vitamin C and E, are publicly discussed to any significant degree (Kahkonen *et al.,* 1999). Polyphenols also appears to have anti-aging and anti-inflammatory properties. Phenol is the simplest aromatic alcohol. Acidity and partial water miscibility are critical for phenol extraction. Phenols are ten orders of magnitude stronger acids than aliphatic alcohols and hence vastly better hydrogen bond donors (Ndoumou *et al.,* 1996).

Polyphenols like the flavonoids and tannins (Sahelian *et al.,* 2006). Polyphenols account for approximately 2% w/w of fresh unfermented cocoa beans. They are

essentially found in the cocoa liquor and powder. Total polyphenol content in cocoa powder, as estimated by the Folin assay, is 5624 mg/100 g. The main polyphenols are flavanols, which include monomers (catechins) and polymers (proanthocyanidins). Phenolic acids, flavonols, some stilbenes, simple phenols and isocoumarins are also present in minor amounts (Othman *et al.,* 2005)

Most natural products can be classified into three major groups such as terpenoids, alkaloids, and phenolic compounds. Phenolic compounds, which are synthesized primarily from products of the shikimic acid pathway, have several important roles in plants. Tannins, lignans, flavonoids, and some simple phenolic compounds serve as defenses against herbivores and pathogens (Kahkonen *et al.,* 1999). Phenolic compound were found in cocoa. Cocoa bean and its products (cocoa liquor, cocoa powder, and dark chocolate) are food sources rich in phenolic compounds. Cocoa beans have a high phenolic content of about 12–18% (dry weight) in unfermented beans. Dreosti (2000) reported that 60% of the total phenolics in raw cocoa beans are flavanol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer). These compounds were reported to be a potential candidate to combat free radicals, which are harmful to our body and food systems (Adamson *et al.,* 1999).

2.2 Molecular Imprinting Technique (MIP)

There are many techniques to extract phenolic compound. The analysis of phenol has to be extracted selectively from the samples, resulting in the requirement of highly selective affinity phases for examples, solid phase extraction (SPE) and membrane technique. This study would separate phenol using molecular imprinting technique using

solid phase extraction. Such materials can be manufactured via molecular imprinting (Bruggemann *et al,* 2003).

Solid phase extraction (SPE) is routinely used in many different areas of analytical chemistry. Some of the main fields are environmental and pharmaceutical analysis where cleaning and concentration of the sample are important steps in the analytical protocol. The growth of SPE has largely been at the expense of liquid–liquid extraction (LLE) where the perceived advantages of SPE over LLE are that it consumes less organic solvents and that a wider range of extraction mechanisms can be utilised. Conventionally, solid phase materials have included reversed phase sorbents, such as C18, C8, normal phases such as silica gel and diol and ion exchange sorbents such as SCX and SAX. For a sorbent to be useful it must enable selective extractions to be achieved. Molecularly imprinted polymers (MIP) potentially offer a higher degree of selectivity than conventional materials which may give an advantage in sample preparation. Although a new concept for analytical chemistry molecular imprinting was introduced nearly 50 years ago by Dickey and others. Reviewing the more recent literature reveals, however, that it is only in the last decade, and especially in the last five years, that the use of molecular imprinting has become established. Molecular imprinting involves the preparation of a polymer with specific recognition sites for certain molecules (Olsen *et al.,* 1998).

The synthesis of MIP takes place by assembly of monomers around a template molecule and subsequent polymerisation using a suitable cross-linker, giving a rigid and robust material. Subsequent removal of template molecules provides a polymer with recognition sites (cavities) allowing specific rebinding of template molecule. The recognition is due to shape and physicochemical properties such as hydrogen bonding, ionic interactions and hydrophobic interactions (Ranstrom *et al.,* 1996). Due to the specific recognition offered by MIPs these materials should be applicable in fields where binding with high selectivity and affinity is required. The binding ability of MIPs can be

likened to that of antibodies in that shape plays an important role in binding. However, at least potentially, MIPs present a number of advantages compared to antibodies. Thus MIPs are easily and rapidly prepared using standard (and well understood) chemical methods, and are stable at high temperatures and in organic solvents. By comparison, immune responses are by nature unpredictable, irreproducible and can require long periods of time to achieve (Sellergen, 1997). Due to their antibody-like behaviour one of the areas of analysis where molecular imprints have been explored is in immunoassay as antibody substitutes. Another area where the applicability of MIPs extensively have been investigated is in HPLC as chiral stationary phases (Mayes *et al.,* 1997). However, the features of molecular imprints are also attractive as sorbents for solid phase extraction (MIP-SPE) as discussed here.This involves the synthesis of cross-linked polymers around a template molecule (the analyte). Once the polymer has been formed the template is removed by washing, leaving an `imprint' of the analyte template. Ideally this gives a sorbent on which highly selective, reversible binding of the analyte can achieved (Stevenson, 1999).

The potential for MIP as SPE sorbents was first reported by Sellergren in 1994. A MIP with recognition sites for the drug pentamidin which is an antiprotozoal drug was synthesised and evaluated for on-line SPE. The MIP was prepared using methacrylic acid as monomer and ethylene glycol dimethacrylate as cross-linker. This combination of monomer and crosslinker has subsequently been used for the synthesis of all of the applications of MIPs for SPE reported to date. Sellergren achieved selective extractions and concentration of samples when the technique was applied to the analysis of biological fluids. A urine sample was spiked with pentamidin and the MIP based extraction resulted in a clean extract and enrichment of the sample to a level where direct detection could be achieved. Despite this demonstration of MIP technology in SPE, it was several more years before the next applications of MIPs in SPE appeared. Following extractions from aqueous samples, cumulative elution curves (where the percentage of organic solvent was varied from 0–100%) were obtained in order to assess

different elution solvents. To achieve quantitative recoveries the presence of an ionic modifier was necessary (Olsen *et al.,* 1998).

Probably the most interesting finding in this study was the importance of selecting the correct ionic modifier for the elution step as this greatly affected the selectivity of the extraction. More recent studies on this type of MIP extended the work to a greater range of propranolol analogs as a means of exploring the selectivity of the approach. In addition this work demonstrated, using radiolabelled propranolol for imprinting, the difficulty of obtaining complete recovery of the template with subsequent leaching detected when trace analysis was attempted. The MIP approach for bioanalysis was also investigated by Muldoon *et al*. An atrazine-derived MIP was used to extract the herbicide from organic extracts from beef liver samples. Optimising the extraction solvent showed chloroform to be the best in terms of recovery and low non-specific binding to the polymer. The chloroform extracts were either directly or subjected to a MIP based SPE procedure prior to quantification. The protocol for SPE consisted of the application of the organic extracts, followed by washes with chloroform to remove lipids and then elution of atrazine with acetonitrile containing 10% acetic acid. MIP-SPE in this application provided good recovery and clean extracts, which lead to improvements in assay precision, accuracy and a lower detection limit for the HPLC method. This was due to removal of interfering components (Olsen *et al.,* 1998).

Performing MIP based extractions from organic solvents for hydrophobic molecules such as atrazine exploited the observation that MIPs offer the best binding in non-polar solvents especially the solvent used during the polymerisation process. A second example of the use of MIP-based SPE for atrazine was presented by Matsui *et al*. This time, however, the MIP- SPE was used for environmental analysis. In contrast to Muldoon *et al*., suspension polymerisation was used which produced bead shaped particles with a uniform particle size distribution. This MIP also allowed the selective extraction of a close structural analog of atrazine (simazine) from a mixture containing

other agrochemicals to be performed. The compounds were applied to the MIP in water and under these conditions it was suspected that simazine was initially bound to the MIP *via* non-specific hydrophobic interactions. Therefore following extraction, the sorbent was subsequently dried and washed with the non-polar dichloromethane allowing selective rebinding of simazine (Olsen *et al.,* 1998).

On the initial application all compounds in the test mixture were retained on the phase but the wash with dichloromethane removed the unwanted impurities whilst selectively leaving simazine on the polymer. High extraction efficiencies (91%) and a 56 fold concentration were achieved. A further example of MIP-SPE was reported by Walshe *et al*.14 who evaluated the application of MIPs for the extraction of 7-hydroxycoumarin. This group investigated variables of the polymerisation process and chose the conditions which produced the most selective polymer (Olsen *et al.,* 1998).

One of the advantages of SPE over LLE is that it does not require the use and subsequent disposal of large volumes of organic solvents. Comparison of MIP-SPE and LLE for quantifying sameridine (an anaesthetic) in plasma was performed by Andersson *et al*. In this application, significant leaching of the template molecule occurred from the polymer leading to interference at the analysis stage. Indeed it was shown that only 91.5% of the template molecule could be removed from the MIP and that leaching during the desorption step occurred. To overcome this they used a MIP based on a structural analog of sameridine. A MIP based assay for sameridine in plasma (involving addition of the polymer to an organic extract) was developed, validated and compared to an already existent LLE method. Comparison of the SPE and LLE method demonstrated equality in terms of accuracy and precision. However, MIP-SPE extracts appeared to be the cleanest suggesting the replacement of the existing LLE method by the selective sorbents provided by MIPs. Rashid *et al* have published studies on a tamoxifenderived MIP. In this study they were able to demonstrate advantages for the MIP over a more conventional C18-bonded and the un-imprinted polymer, with high, and reproducible,

recoveries of the target analytes. In addition the authors obtained a MIP that did not show any measurable degree of bleeding of the template (Olsen *et al.,* 1998).

The use of MIPs for SPE is at an early stage and several successful approaches in bioanalysis and environmental analysis have been reported indicating the potential of the concept. However, a number of problems, particularly with regard to template leaching must be solved before the full utilisation of MIPs can be realised in the sample preparation arena (Olsen *et al.,* 1998).

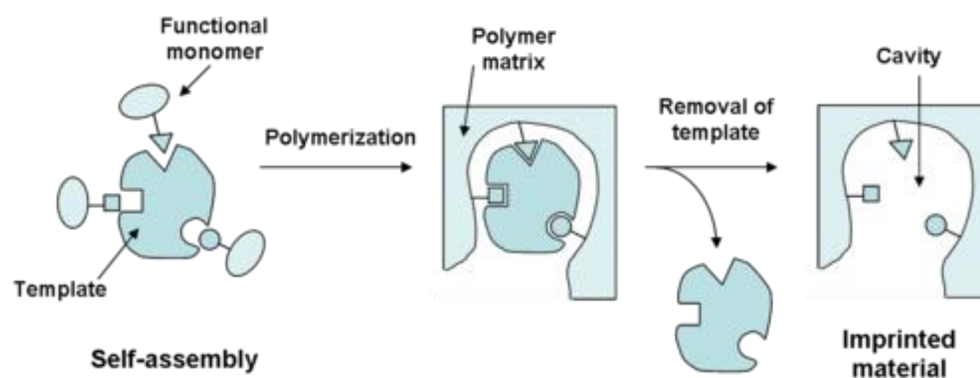

Figure 2.1 Molecular Imprinting Technique (MIP)

2.3 Solid Phase Extraction (SPE)

Solid-phase extraction (SPE) has become the method of choice in many laboratories for the analysis of complex samples. Recently, highly selective extraction based on antibody columns or molecular imprinted polymers (MIPs) has been developed. To date biological antibodies have shown better specificity but MIPs are easier to produce.SPE is a developing area for application of MIP technology. Many

analytical procedures are based on the use of liquid-liquid extraction, solid-phase extraction (SPE) or combinations of these followed by an analytical separation method, typically high performance liquid chromatography (HPLC), gas chromatography (GC) or capillary electrophoresis (CE). Method selection for a particular problem is a matter of personal choice, based on experience and techniques available, as well as analyte and matrix properties. Nonetheless the popularity of SPE has increased in recent years as it is easily automated and a wide range of phases is available. It is also regarded as environmentally friendlier as large volumes of solvents are not used as in liquid liquid extraction (Stevenson, 1999).

Commercially available phases for SPE based on silica and bonded silicas have been used for a wide range of analytes. Early problems with batch-to-batch variation in analyte recovery inhibited their use but these have been addressed by manufacturers. One of the biggest problems was the presence of residual silanols on the most popular reversed phase materials. These could give separations dependent on more than one mechanism of separation and greater vulnerability to variations between different batches. SPE columns based on polymers have been developed to overcome the uncertainty caused by such secondary interactions (Stevenson, 1999).

The difficulty and cost of obtaining biological antibodies has led to attempts to synthesise antibody mimics in the chemistry laboratory. One such approach has been the development and evaluation of molecularly imprinted polymers (MIPs). This involves the synthesis of cross-linked polymers around a template molecule (the analyte). Once the polymer has been formed the template is removed by washing, leaving an `imprint' of the analyte template. Ideally this gives a sorbent on which highly selective, reversible binding of the analyte can achieved. There have been two main approaches to the synthesis of MIPs. After polymerisation they hydrolysed the sugar moiety and used the polymer for selective binding. This approach is usually referred to as covalent molecular imprinting. They used a monomer such as methacrylic acid along with a cross-linker

such as ethylene glycol dimethacrylate mixed with the template (analyte molecule). After polymerisation the analyte is washed out of the polymer leaving a cavity which can selectively bind the template (Stevenson, 1999).

The use of MIPs for solid phase extraction is the topic of this article. The main perceived advantage of MIPs over biological antibodies for SPE is the ease with which they can be obtained and the consequent lower cost and speed. One of the main disadvantages with the MIP approach to SPE is the difficulty in removing all of the template analyte molecule. Even after extensive washing it has proven difficult to achieve this. This leads to leaching of the analyte in actual samples being processed and subsequent inaccurate results. Hence retention in the polymer and subsequent leaching of even a fraction of a per cent of the template is very significant. This problem has been tackled by using a structural analogue to the analyte of interest as the template. It relies on the fact that the MIP will have some affinity to closely related compounds just as many biological antibodies often do. If the template continues to leach out this does not matter as long as it can be separated by the chromatographic end step. It must also separate from the internal standard and any metabolites if these are also to be measured. The selection of washing and elution steps is crucial for optimisation of selectivity when developing a MIP based extraction procedure. With many procedures it has been found that optimum selectivity is found if the solvent in which the polymer was formed is used for retention and elution studies (Stevenson, 1999).

For the MIP approach to be of use in small-scale analytical sample preparation there must be a demonstrable over using commercially available SPE columns (Stevenson, 1999). Solid phase extraction is used to separate compounds of interest from impurities in three ways which are selective extraction, selective washing and selective elution. The SPE process provides samples that are in solution, free of interfering matrix components, and concentrated enough for detection.

Solid phase extraction (SPE) is an increasingly useful sample preparation technique. With SPE, many of the problems associated with liquid/liquid extraction can be prevented, such as incomplete phase separations, less-than-quantitative recoveries, use of expensive, breakable specialty glassware, and disposal of large quantities of organic solvents. SPE is more efficient than liquid/liquid extraction, yields quantitative extractions that are easy to perform, is rapid, and can be automated. SPE is used most often to prepare liquid samples and extract semivolatile or nonvolatile analytes, but also can be used with solids that are pre-extracted into solvents. SPE products are excellent for sample extraction, concentration, and cleanup. They are available in a wide variety of chemistries, adsorbents, and sizes. Selecting the most suitable product for each application and sample is important. In this study, I am using Solid Phase Extraction Guide in order to select the best silica to be used and the suitable phase for extraction phase. In conclusion in selecting the suitable silica and phase, silica C_{18} and reversed phase SPE have been chosen according to the table SPE phase types.

Table 2.1: SPE Phase Types

Silica-Based Packing	Characteristics	Used
LC-18	octadecyl bonded, endcapped silica	For reversed phase extraction of nonpolar to moderately polar compounds, such as antibiotics, barbiturates,benzodiazepines, caffeine, drugs, dyes, essential oils, fat soluble vitamins, fungicides, herbicides, pesticides, hydrocarbons, parabens, phenols, phthalate esters, steroids, surfactants, theophylline, and water soluble vitamins.

Figure 2.2 Sep-Pak Vac 35cc. (silica C_{18} 10g)

2.3.1 Reversed Phase SPE

Reversed phase separations involve a polar or moderately polar sample matrix (mobile phase) and a nonpolar stationary phase. The analyte of interest is typically mid- to nonpolar. Several SPE materials, such as the alkyl- or aryl-bonded silicas (LC-18, ENVI-18, LC-8, ENVI-8, LC-4, and LC-Ph) are in the reversed phase category. Here, the hydrophilic silanol groups at the surface of the raw silica packing (typically 60Å pore size, 40µm particle size) have been chemically modified with hydrophobic alkyl or aryl functional groups by reaction with the corresponding silanes.

Retention of organic analytes from polar solutions (e.g. water) onto these SPE materials is due primarily to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the silica surface. These nonpolar-nonpolar attractive forces are commonly called van der Waals forces, or dispersion forces. To

elute an adsorbed compound from a reversed phase SPE tube or disk, use a nonpolar solvent to disrupt the forces that bind the compound to the packing. LC-18 and LC-8 are standard, monomerically bonded silicas.

The primary retention mechanisms for compounds on the SPE materials are described above. For the bonded silicas, it is possible that secondary interactions will occur. For reversed phase bonded silicas, the primary retention mechanism involves nonpolar interactions. However, because of the silica particle backbone, some polar secondary interactions with residual silanols such as those described for normal phase SPE could occur. If a nonpolar solvent does not efficiently elute a compound from a reversed phase SPE packing, the addition of a more polar solvent (e.g. methanol) may be necessary to disrupt any polar interactions that retain the compound. In these cases, methanol can hydrogen-bond with the hydroxyl groups on the silica surface, thus breaking up any hydrogen bonding that the analyte may be incurring. The silanol group at the surface of the silica, Si-OH, can also be acidic, and may exist as an Si-O- group above pH 4. As a result, the silica backbone may also have cation exchange secondary interactions, attracting cationic or basic analytes of interest. In this case, a pH adjustment of the elution solvent may be necessary to disrupt these interactions for elution (acidic to neutralize the silanol group, or basic to neutralize the basic analyte). This can be done by using acidic methanol (98% MeOH:2% concentrated HCl) or basic methanol (98% MeOH:2% concentrated NH4OH), or by mixtures of these with a more nonpolar, methanol-miscible solvent.

For reversed phase SPE procedures on bonded silicas, if trapping the analyte in the tube is desired, the pH of the conditioning solution and sample (if mostly or entirely aqueous) should be adjusted for optimum analyte retention. If the compound of interest is acidic or basic you should, in most cases, use a pH at which the compound is not charged. Retention of neutral compounds (no acidic or basic functional groups) usually is not affected by pH. Conversely, you can use a pH at which the unwanted compounds

in the sample are retained on the SPE packing, but the analyte of interest passes through unretained. Secondary hydrophilic and cation exchange interactions of the analyte can be used for retention at a proper pH.

Solid phase extraction is used to separate compounds of interest from impurities in three ways. First step is selective extraction. Select an SPE sorbent that will bind selected components of the sample either the compounds of interest or the sample impurities. The selected components are retained when the sample passes through the SPE tube or disk (the effluent will contain the sample minus the adsorbed components). Then, either collect the adsorbed compounds of interest through elution, or discard the tube containing the extracted impurities. Second step is selective washing. The compounds of interest and the impurities are retained on the SPE packing when the sample passes through the impurities are rinsed through with wash solutions that are strong enough to remove them, but weak enough to leave the compounds of interest behind. Final step is selective elution. The adsorbed compounds of interest are eluted in a solvent that leaves the strongly retained impurities behind.

The SPE process provides samples that are in solution, free of interfering matrix components, and concentrated enough for detection. This is done in five steps. Firstly, select the proper SPE tube. An SPE disk is recommended for large volume samples, samples containing high amounts of particulates, or when a high flow rate is required during sampling. It is also depends on the amount of the sample that we want to extract. Secondly is condition the SPE tube. To condition the SPE tube packing, rinse it with up to one tube-full of solvent before extracting the sample. Reversed phase type silicas and nonpolar adsorption media usually are conditioned with a water-miscible organic solvent such as methanol, followed by water or an aqueous buffer. Methanol wets the surface of the sorbent and penetrates bonded alkyl phases, allowing water to wet the silica surface efficiently. Sometimes a pre-conditioning solvent is used before the methanol step. This solvent is usually the same as the elution solvent, and is used to remove any impurities

on the SPE tube that could interfere with the analysis, and may be soluble only in a strong elution solvent.

Thirdly, accurately transfer the sample to the tube or reservoir, using a volumetric pipette or micropipette. The sample must be in a form that is compatible with SPE. Total sample volume can range from microliters to liters. When excessive volumes of aqueous solutions are extracted, reversed phase silica packings gradually lose the solvent layer acquired through the conditioning process. This reduces extraction efficiency and sample recovery. For samples >250mL, add small amounts of water-miscible solvents (up to 10%) to maintain proper wetting of reversed phase packings. Maximum sample capacity is specific to each application and the conditions used. If recoveries are low or irreproducible, test for analyte breakthrough using the following technique: Attach two conditioned SPE tubes of the same packing together using an adapter. Pass the sample through both tubes. When finished, detach each tube and elute it separately. If the analyte is found in the extract of the bottom tube, the sample volume is too great or bed weight is too small, resulting in analyte breakthrough. To enhance retention of appropriate compounds on the packing, and elution or precipitation of unwanted compounds, adjust the pH, salt concentration, and/or organic solvent content of the sample solution. To avoid clogging SPE tube frits or the SPE disk, pre-filter or centrifuge samples prior to extraction if possible. Slowly pass the sample solution through the extraction device, using either vacuum or positive pressure. The flow rate can affect the retention of certain compounds. Generally, the flow rate should not exceed 2mL/min for ion exchange SPE tubes, 5mL/min for other SPE tubes, and may be up to 50mL/min for disks. Dropwise flow is best, when time is not a factor.

Fourthly, wash the packing. If compounds of interest are retained on the packing, wash off unwanted, unretained materials using the same solution in which the sample was dissolved, or another solution that will not remove the desired compounds. Usually no more than a tube volume of wash solution is needed, or 5-10mL for SPE disks. To

remove unwanted, weakly retained materials, wash the packing with solutions that are stronger than the sample matrix, but weaker than needed to remove compounds of interest. A typical solution may contain less organic or inorganic salt than the final eluant. It also may be adjusted to a different pH. Pure solvents or mixtures of solvents differing sufficiently in polarity from the final eluant may be useful wash solutions. If you are using a procedure by which compounds of interest are not retained on the packing, use about one tube volume of the sample solvent to remove any residual, desired components from the tube, or 5 10mL to remove the material from a disk. This rinse serves as the elution step to complete the extraction process in this case.

Finally, rinse the packing with a small volume (typically 200µL to 2mL depending on the tube size, or 5-10mL depending on the disk size) of a solution that removes compounds of interest, but leaves behind any impurities not removed in the wash step. Collect the eluate and further prepare as appropriate. Two small aliquots generally elute compounds of interest more efficiently than one larger aliquot. Recovery of analytes is best when each aliquot remains in contact with the tube packing or disk for 20 seconds to 1 minute. Slow or dropwise flowrates in this step are beneficial.

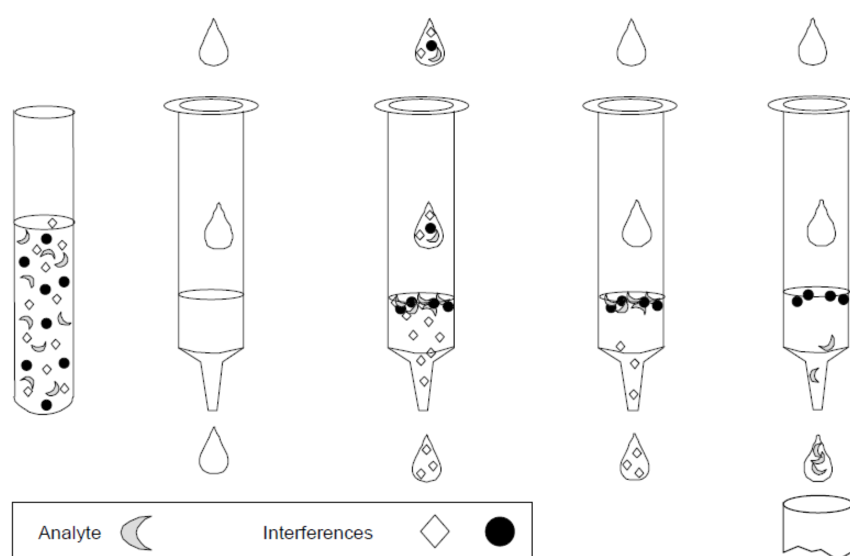

Figure 2.3 Solid Phase Extraction Steps

2.4 Suspension Polymerization

Suspension polymerization is the established polymerization method to prepare polymer beads with the size region from micron to thousand microns. In the suspension polymerization method, it is important to investigate how to make the suspension stable and how to control the polymer bead size. In the method for controlling polymer bead size, there are the soft type of technique by adjusting physical properties of liquids concerned and the hard type of technique by investigating the operating conditions as the geometry of reactor and the mixing condition. By suspension polymerization, composite particles with various structures can be prepared (Tanaka, 2005).

A polymerization process in which the monomer, or mixture of monomers are dispersed by mechanical agitation in a liquid phase, usually water, in which the monomer droplets are polymerized while they are dispersed by continuous agitation.

2.5 Hydrolysis

Hydrolysis literally means reaction with water. It is a chemical process in which a molecule is cleaved into two parts by the addition of a molecule of water. One fragment of the parent molecule gains a hydrogen ion (H^+) from the additional water molecule. The other group collects the remaining hydroxyl group (OH^-).

Hydrolysis is a chemical reaction during which molecules of water (H_2O) are split into hydroxide cations (H^+) (conventionally referred to as protons) and hydroxide

anions (OH^-) in the process of a chemical mechanism. It is the type of reaction that is used to break down certain polymers, especially those made by step-growth polymerization. Such polymer degradation is usually catalysed by either acid such as concentrated sulfuric acid (H_2SO_4), or alkali, sodium hydroxide (NaOH) attack, often increasing with their strength or pH. Hydrolysis is distinct from hydration. In hydration, the hydrated molecule does not "lyse" (break into two new compounds). It should not be confused with hydrogenolysis, a reaction of hydrogen (Freifelder *et al.,* 1987)

Hydrolysis is a chemical process in which a certain molecule is split into two parts by the addition of a molecule of water. One fragment of the parent molecule gains a hydrogen ion (H^+) from the additional water molecule. The other group collects the remaining hydroxyl group (OH^-). The most common hydrolysis occurs when a salt of a weak acid or weak base (or both) is dissolved in water. Water autoionizes into negative hydroxyl ions and positive hydrogen ions. The salt breaks down into positive and negative ions. For example, sodium acetate dissociates in water into sodium and acetate ions. Sodium ions react very little with hydroxyl ions whereas acetate ions combine with hydrogen ions to produce neutral acetic acid, and the net result is a relative excess of hydroxyl ions, causing a basic solution.

However, under normal conditions, only a few reactions between water and organic compounds occur. Generally, strong acids or bases must be added in order to achieve hydrolysis where water has no effect. The acid or base is considered a catalyst . They are meant to speed up the reaction, but are recovered at the end of it.

Acid–base-catalyzed hydrolyses are very common; one example is the hydrolysis of amides or esters. Their hydrolysis occurs when the nucleophile (a nucleus-seeking agent, e.g., water or hydroxyl ion) attacks the carbon of the carbonyl group of the ester or amide. In an aqueous base, hydroxyl ions are better nucleophiles than dipoles such as

water. In acid, the carbonyl group becomes protonated, and this leads to a much easier nucleophilic attack. The products for both hydrolyses are compounds with carboxylic acid groups (Freifelder *et al.,* 1987).

Perhaps the oldest example of ester hydrolysis is the process called saponification. It is the hydrolysis of a triglyceride (fat) with an aqueous base such as sodium hydroxide (NaOH). During the process, glycerol, also commercially named glycerin, is formed, and the fatty acids react with the base, converting them to salts. These salts are called soaps, commonly used in households.

Moreover, hydrolysis is an important process in plants and animals, the most significant example being energy metabolism and storage. All living cells require a continual supply of energy for two main purposes: for the biosynthesis of small and macromolecules, and for the active transport of ions and molecules across cell membranes. The energy derived from the oxidation of nutrients is not used directly but, by means of a complex and long sequence of reactions, it is channeled into a special energy-storage molecule, adenosine triphosphate (ATP) (Freifelder *et al.,* 1987).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Materials

In this study, methacrylic acid (MAA) is used as a monomer and ethylene glycol dimethacrylate (EGDMA) as a cross-linker. Phenol is used in this study as a template. The initiator used is dimethylaminophenol (DMPAP) while solvent used are methanol.

3.2 Methodology

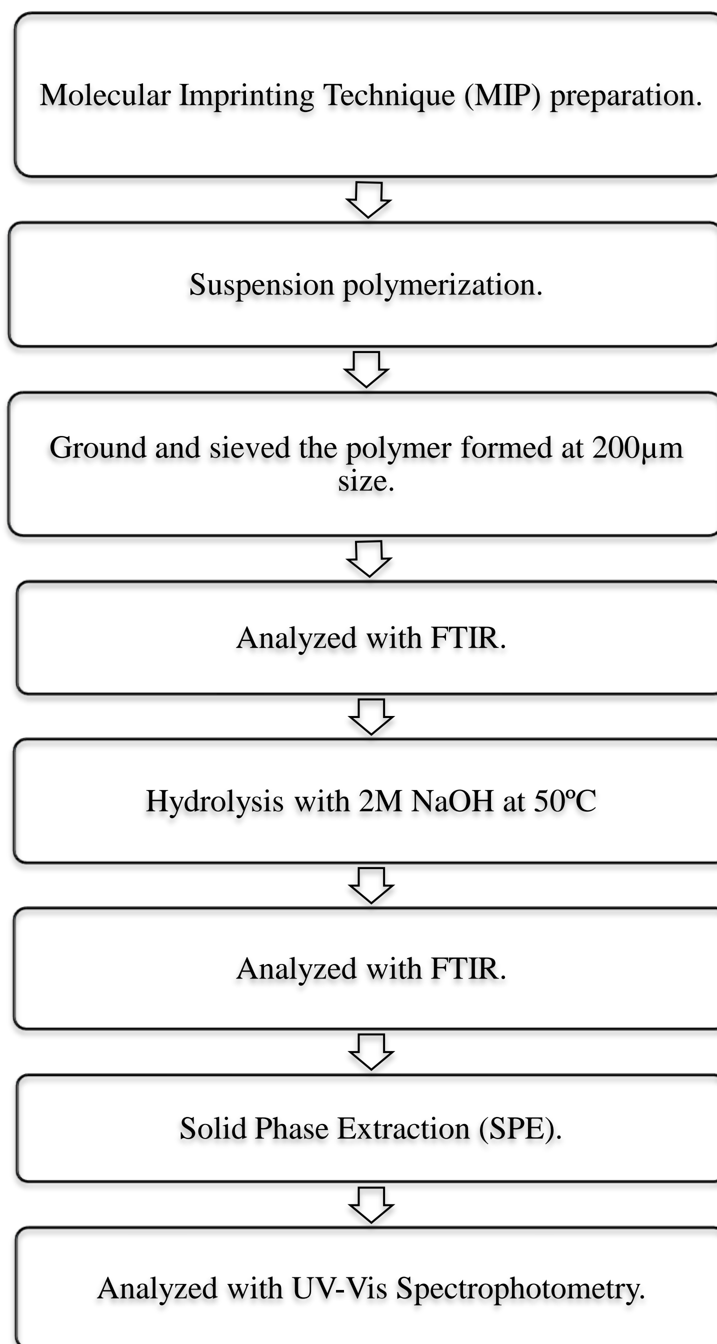

Figure 3.1 Flow of Methodology.

3.2.1 Preparation of MIP

Five different compounds were generated according to the recipes listed in Table 3.1. First of all, the methanol solvent was filled into a vial. This was followed by the addition of the liquid functional monomer, the liquid cross-linker and the initiator using for all three components a balance for determining the exact weight. For the MIP, additionally the template was weighed and added to the mixture (Bruggemann *et al*., 2003). This method is repeated without template as well to observe the presence of phenol in polymer.

Table 3.1: Polymer recipes (Bruggemann *et al*., 2003).

Function	Compound	Amount ratio	Mass (g)
Solvent	Methanol	-	5ml
Monomer	MAA	4	1.148
Cross-linker	EDGMA	12	7.929
Initiator	DMPAP	0.17	0.08
Template	Phenol	1	0.6

Molecular imprinting involves the preparation of a polymer with specific recognition sites for certain molecules. The synthesis of MIP takes place by assembly of monomers around a template molecule and subsequent polymerization using a suitable cross-linker, giving a rigid and robust material. A monomer such as methacrylic acid (MAA) along with a cross-linker such as ethylene glycol dimethacrylate (EDGMA) mixed with the template (analyte molecule). After polymerisation the analyte is washed out of the polymer leaving a cavity which can selectively bind the template. Subsequent removal of template molecules provides a polymer with recognition sites (cavities) allowing specific rebinding of template molecule. The recognition is due to shape and physicochemical properties such as hydrogen bonding, ionic interactions and

hydrophobic interactions. Due to the specific recognition offered by MIPs these materials should be applicable in fields where binding with high selectivity and affinity is required (Stevenson, 1999). Elution of the template was investigated via UV-detection at 320 nm to observe the absorbance on the effects of adsorbent amount and concentration of phenol solution (Bruggemann *et al*., 2003).

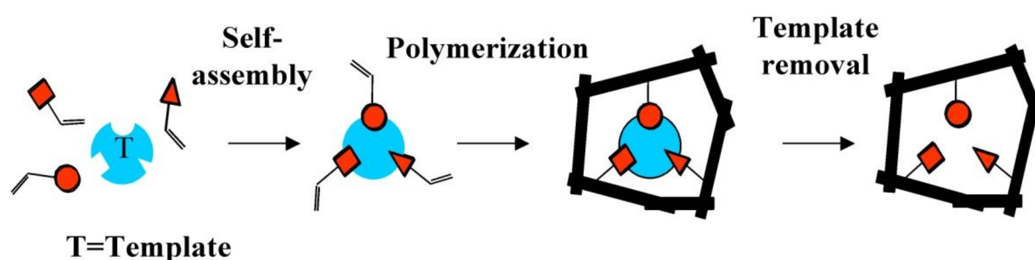

Figure 3.2 Molecular imprinting polymer technique.

3.2.2 Suspension polymerization

Chronologically, the first polymerization method employed to synthesis a MIP was based on the suspension polymerization method. It is most widely applied by groups working on imprinting because of its simplicity and universality. In suspension polymerization, the mixture was sparged with nitrogen gas for 10 to 30 min to remove oxygen in the vial. Then, the mixture was then polymerized under ultraviolet (UV) light for two hours. This is because the initiator that used is DMPAP which is UV initiator type. After two hours, polymer formed in a form of crystal. The resulting polymer was ground into particles of 200 μm diameter using a mortar and pestle. Then, the polymer must be hydrolysed to break down the polymer and catalysed by 2M sodium hydroxide (NaOH), often increasing with their strength or pH. After that, the polymer was analyzed using Fourier Transform Infrared Spectroscopy (FTIR).

Basically all the components, which are mainly the template molecule, monomer, cross-linker, initiator and solvent are mixed well and proceed to polymerize ultraviolet (UV) radiation. This process is time consuming and wasteful since a lot of the polymer is lost in the process of grinding and filtration to obtain fine particles. It may also produce areas of heterogeneity in the polymeric matrix resulting from the lack of control during polymerization process, particularly when UV initiation is used (Olsen *et al.,* 1998).

3.2.3 Solid phase extraction process

Solid phase extraction is used to separate compounds of interest from impurities in three ways. For selective extraction, select an SPE sorbent that will bind selected components of the sample either the compounds of interest or the sample impurities. The selected components are retained when the sample passes through the SPE tube (the effluent will contain the sample minus the adsorbed components). Then, either collect the adsorbed compounds of interest through elution, or discard the tube containing the extracted impurities.

For selective washing, the compounds of interest and the impurities are retained on the SPE packing when the sample passes through; the impurities are rinsed through with wash solutions that are strong enough to remove them, but weak enough to leave the compounds of interest behind. For selective elution, the adsorbed compounds of interest are eluted in a solvent that leaves the strongly retained impurities behind. Target compound are analyzed using UV-Vis Spectroscopy and FTIR (Garcia *et al.,* 2005).

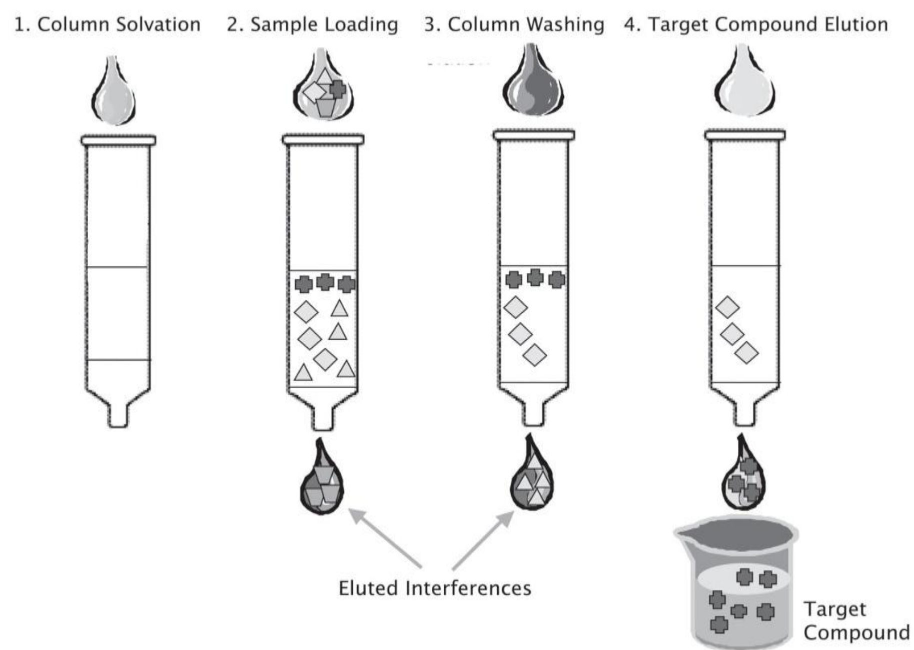

Figure 3.3 Solid Phase Extraction.

CHAPTER 4

RESULT AND DISCUSSION

From Figure 4.1 and Figure 4.2, the differences clearly observed between the polymer with and without template. It showed the presence of template in adsorption of phenol. In Figure 4.1, there are two layers of absorbance can be observed, which means for the first layer, the absorbance occurred pass through inside the absorbent while the second layer means the absorbance occurred only at the surface of adsorbent because the adsorbent was too compact with template. The Figure 4.2 shows that the absorbance become optimum at 300mg/L with template. The higher adsorbent amount and concentration gave the higher percentage of absorbance.

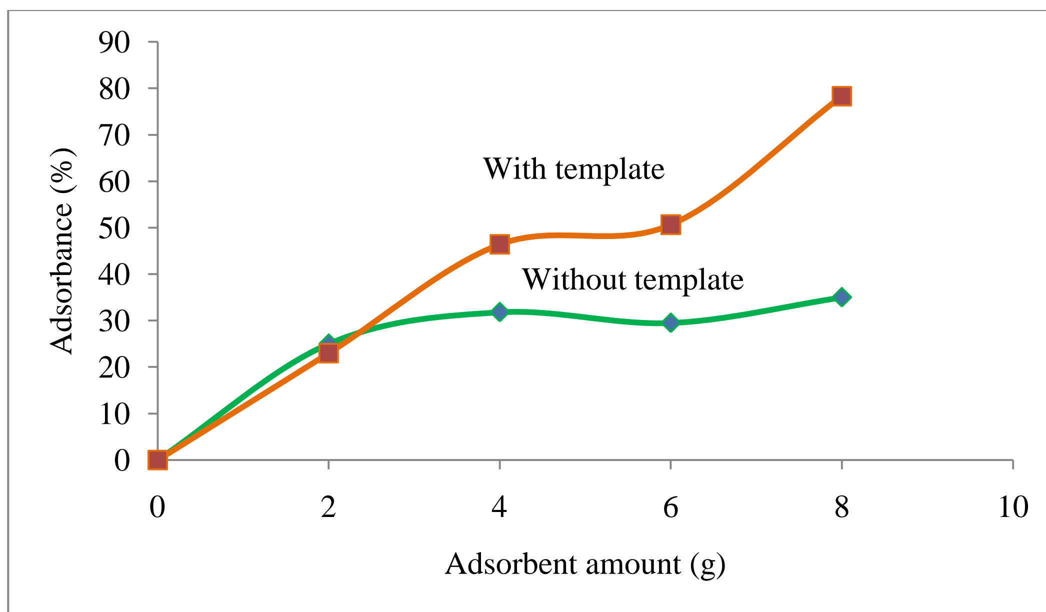

Figure 4.1 Effect of adsorbent amount for polymer.

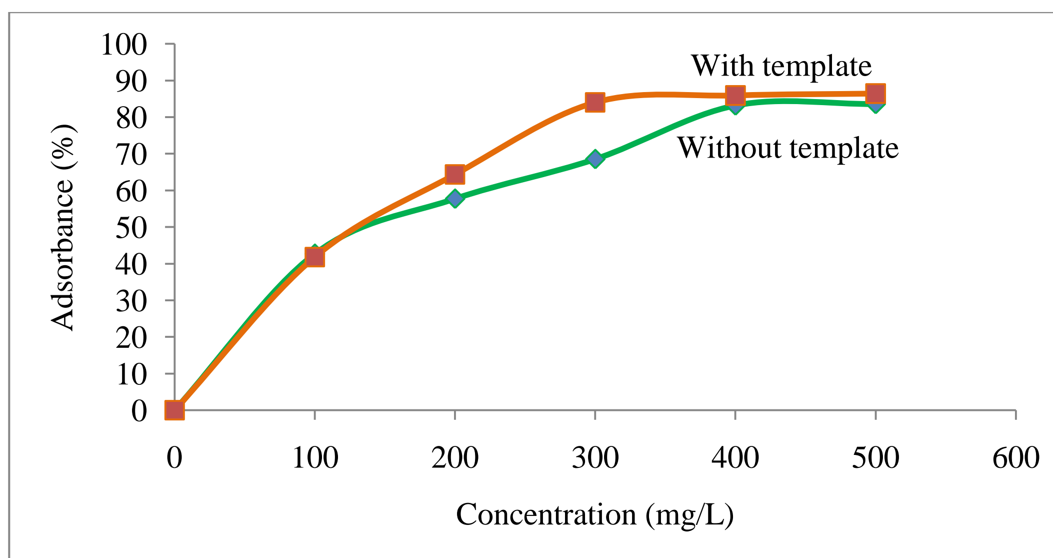

Figure 4.2 Effect of concentration of phenol solution for polymer.

The objective of constructing Figure 4.3 and Figure 4.4 is to study the selectivity of adsorption of phenol either using MIP polymer and control polymer which is silica C_{18}. In Figure 4.3, there are two layers of absorbance can be observed, which means for the first layer is at 0g to 4g of adsorbent amount, the absorbance occurred pass through inside the absorbent while the second layer, 6g to 8g of adsorbent amount, means the absorbance occurred only at the surface of adsorbent because the adsorbent was too

compact with template. The Figure 4.4 shows that the absorbance become optimum at 300mg/L with template. A higher selectivity of target molecule proved when performing the extraction using MIP polymer.

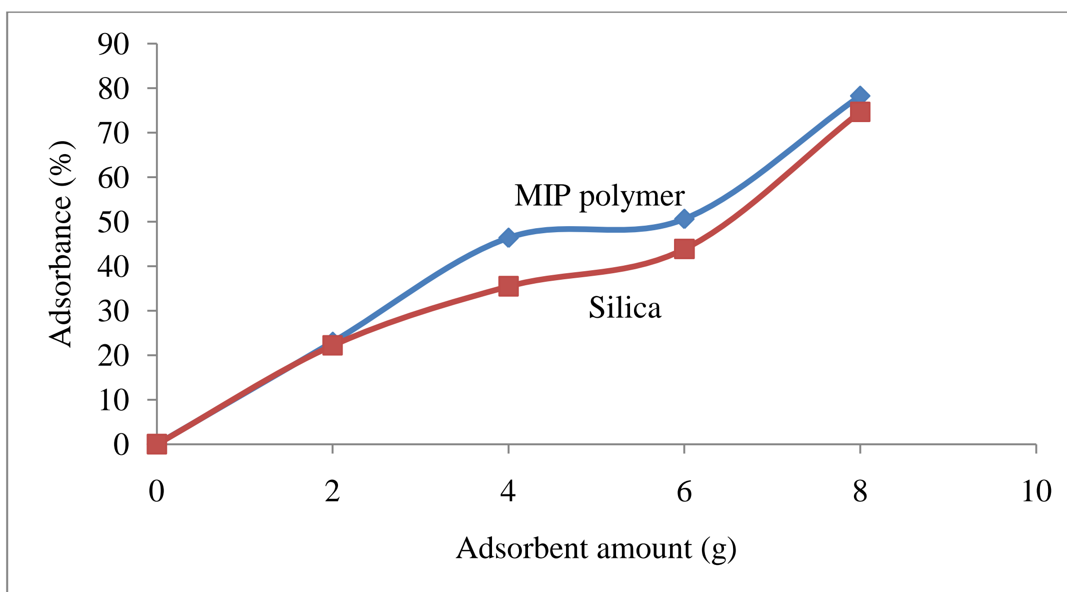

Figure 4.3 Effect of adsorbent amount on absorbance difference between polymer and silica.

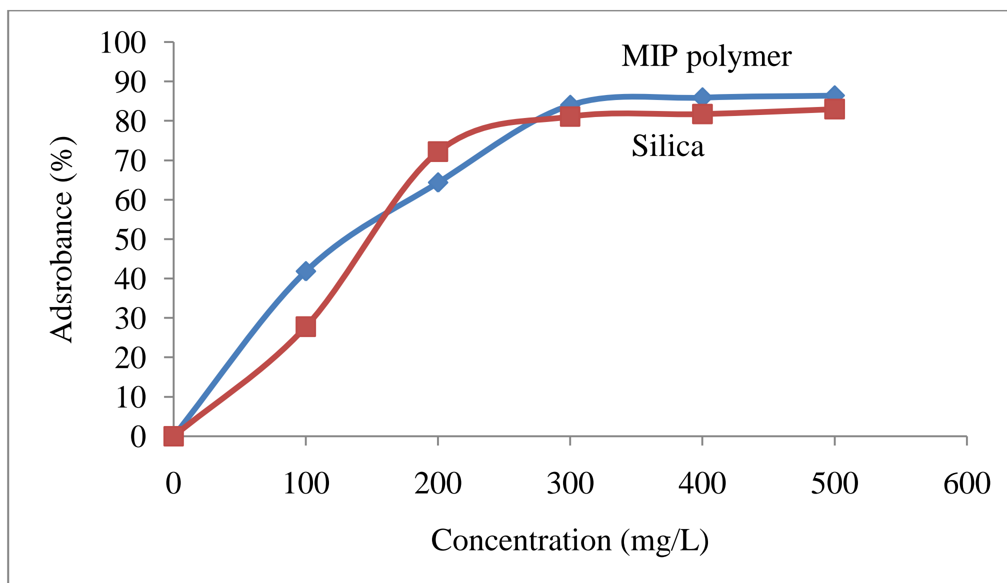

Figure 4.4 Effect of concentration of phenol solution on absorbance difference between polymer and silica.

The experimental results from Figure 4.5 and Figure 4.6 clearly showed that higher adsorbent amount and higher concentration of phenol solution gave higher absorbance capacity of phenol. A higher selectivity of target molecule proved when performing the extraction using polymer. When using this polymer as adsorbent in SPE, it showed higher affinity towards their respective template molecules, compared to the control polymer which is silica C_{18}. In this experiment, the result showed that methanol is an effective solvent for extraction of phenolic compound.

The polymer was quite difficult to prepare which is the mixture must be well polymerized to get in crystal form. Under the optimum conditions there was a clear difference between the absorbance of the MIP and the control polymer. MIP is prepared using small amount of a template molecule and if this template is not completely removed during washing then leaching of template during the subsequent SPE can interfere with trace analysis. This problem was overcome by used a structural analogue of the analyte for the preparation of the polymer. In addition, the structural analogue must be carefully chosen so as not to interfere with the analysis of the test compound but such that it can achieve selective binding in the MIP cavities.

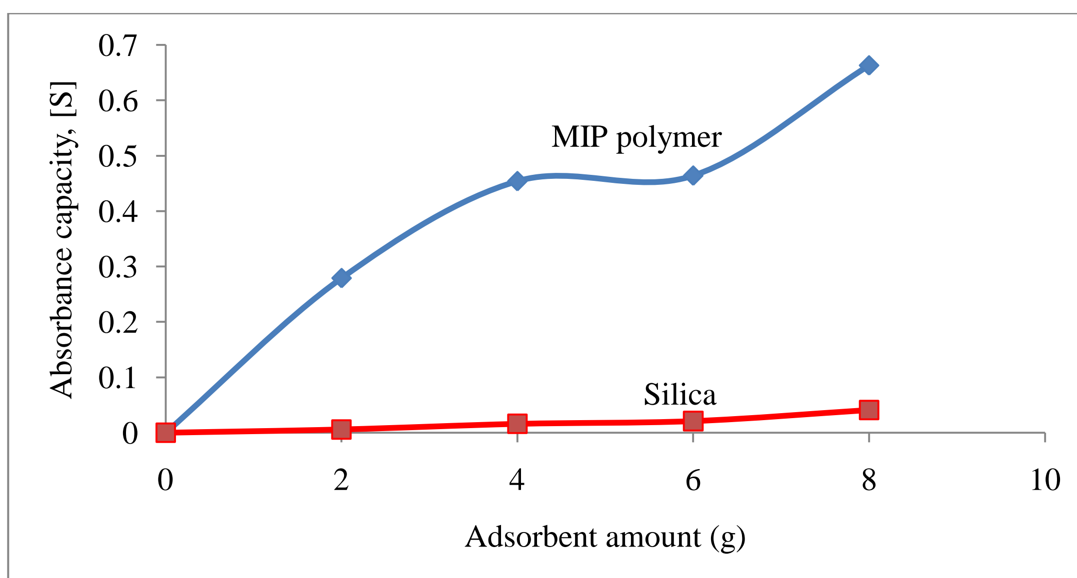

Figure 4.5 Difference of absorbance capacity between polymer and silica on the effect of adsorbent amount.

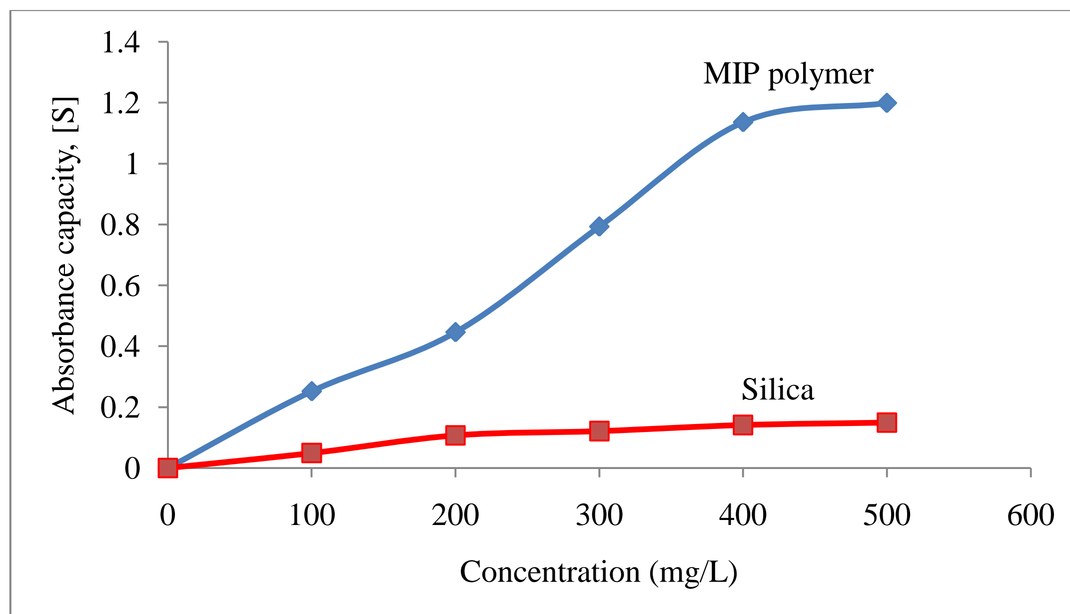

Figure 4.6 Difference of absorbance capacity between polymer and silica on the effect of concentration.

Fourier transform spectroscopy (FTIR) is a technique to detect and identify the unknown material in a sample or specimen. The obtained molecularly imprinted polymers were analyzed by FTIR. There are some error occurred while detecting of phenol using FTIR. Many peaks observed while reading the peaks because some noise may disturb the detection of phenol. In Figure 4.7, the comparison made to observe the differences of polymer, template and polymer with template before and after hydrolysis but the error occurred so that it gave difficulties in reading the peaks. So, to get the peaks, Figure 4.7 was expanding to Figure 4.8. In this experiment, phenol was detected using FTIR as shown in Figure 4.8. The technique works on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A molecule that is exposed to infrared rays absorbs infrared energy at frequencies which are characteristic to that molecule. During FTIR analysis, a spot on the specimen is subjected to a modulated IR beam. In Figure 4.8, it showed that the detection of template in a sample. The specimen's transmittance and reflectance of the infrared rays at different frequencies is translated into an IR absorption plot consisting of reverse peaks. The resulting FTIR spectral pattern is then analyzed and matched with known signatures of identified materials in the FTIR library.

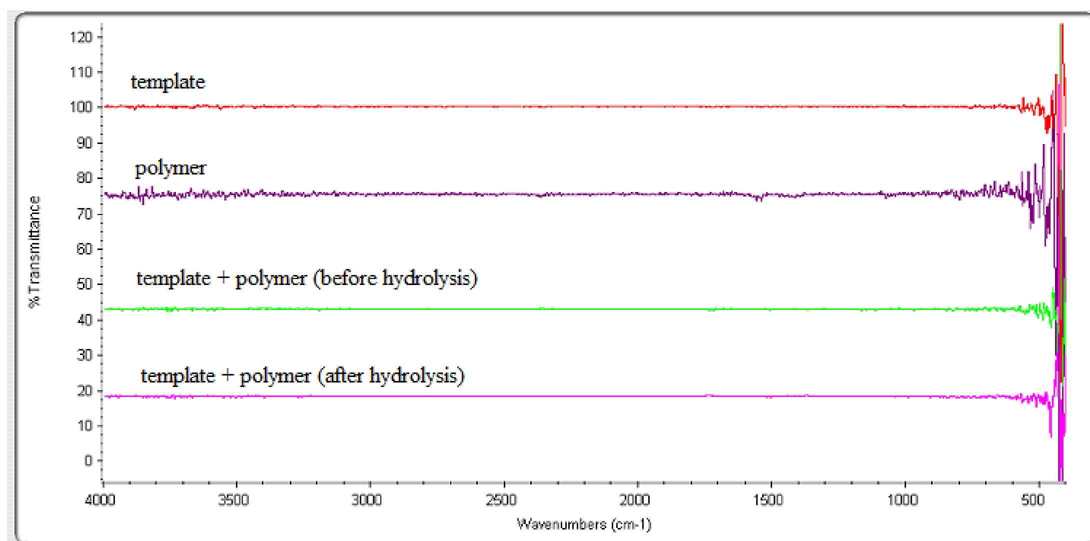

Figure 4.7 Differences of detection of phenol using FTIR.

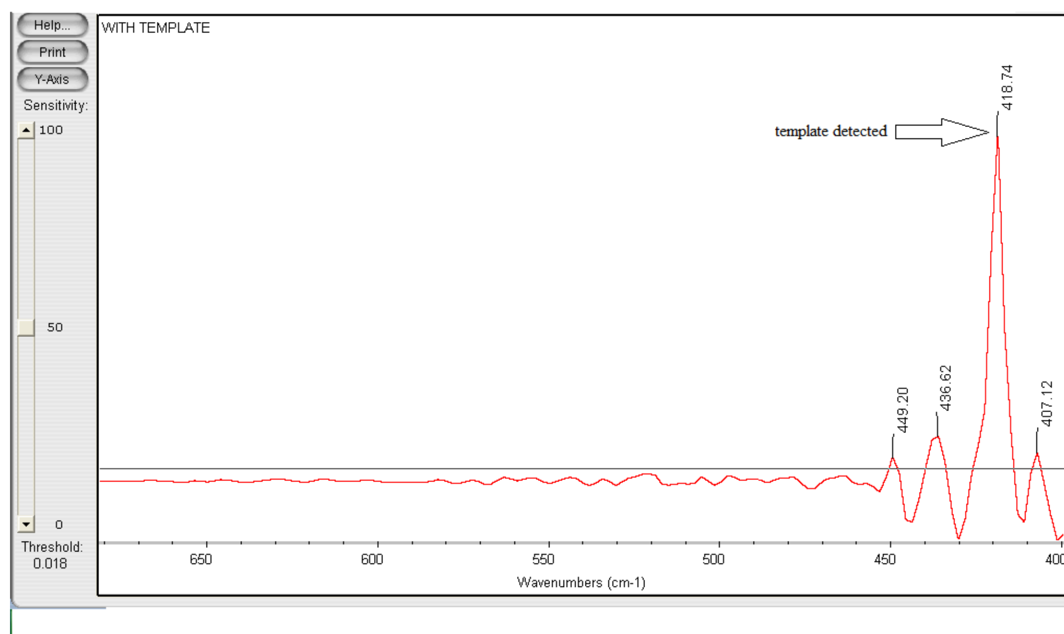

Figure 4.8 Detection of phenol by FTIR.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the objectives of this research were achieved. The study of performance of molecular imprinted polymer (MIP) in solid phase extraction (SPE) process and determination of formulation for preparing of MIP particle and also to analyze the absorbance differences between polymer and silica have been covered.

Methanol proved to be the best solvent for extracting antioxidant from cocoa by products. . Methacrylic acid (MAA) as well as ethyleneglycol dimethacrylate (EGDMA) were chosen as reliable functional monomer and cross-linker, respectively. It was expected that MAA would interact non-covalently with the three template molecules via hydrogen bonds. The use of MIPs for SPE is at an early stage and several successful approaches in bioanalysis and environmental analysis have been reported indicating the potential of the concept. SPE is a developing area for application of MIP technology. For the MIP approach to be of use in small-scale analytical sample preparation there must be a demonstrable benefit over using commercially available SPE columns.

MIP do have the advantage over bioanalysis that they are more stable to harsher conditions of pH, organic solvents, pressure and temperature. The problem of template

leakage with MIP is partly circumvented by using a structural analogue, but only succeeds because of a lack of the very specificity that we are aiming to achieve.

The advantages of MIP for SPE is the ease with which they can be obtained and the consequent lower cost and time, while the disadvantage is the difficulty in removing all of the template analyte molecule. The crucial steps for optimization selectivity were the selection of washing and elution steps. A higher selectivity of target molecule proved when performing the extraction using polymer.

The successful preparation of molecularly imprinted polymers for solid phase extraction provides an innovative opportunity for the development of advanced adsorption antioxidants in plant. The experimental results clearly showed that higher adsorbent amount and higher concentration of phenol solution gave higher absorbance. The optimum adsorbent amount in this experiment is 5g while the optimum concentration of phenol is 300mg/L. A higher selectivity of target molecule proved when performing the extraction using polymer.

5.2 Recommendation

In order to improve this research, there are several things should be stress out in the future. Firstly, if we want to extract the phenolic compound from the plant such as cocoa, the concentration of phenol solution should be smaller since the plant is small in size and of course the phenolic compound amount in plant is smaller. Furthermore, the parameter should be added for future research to study more effects of absorption of phenol. Besides, while analyzed using FTIR, there were some disturbance occur that affect the reading of peak and it was difficult to detect the presence of phenol. So for future experiment, avoid any disturbance that can affect the reading of peaks.in order to get the desire result.

Improved methods of synthesis will allow production of MIP with better selectivity. However, the technique must still remain simple or it will not be an improvement on current SPE methods other than in a few very specific applications where conventional methods are not successful. In this case, if available, offer a feasible alternative. The application of MIP in other areas is also worthy of further study.

CHAPTER 6

REFERENCES

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APPENDIX A

Experimental Result

Table A.1: Effect of adsorbent amount for silica.

Adsorbent amount (g)	Before	After	% Absorbance
Blank	0	0	0
2	0.007	0.009	22.2
4	0.02	0.031	35.48
6	0.032	0.057	43.86
8	0.019	0.075	74.7

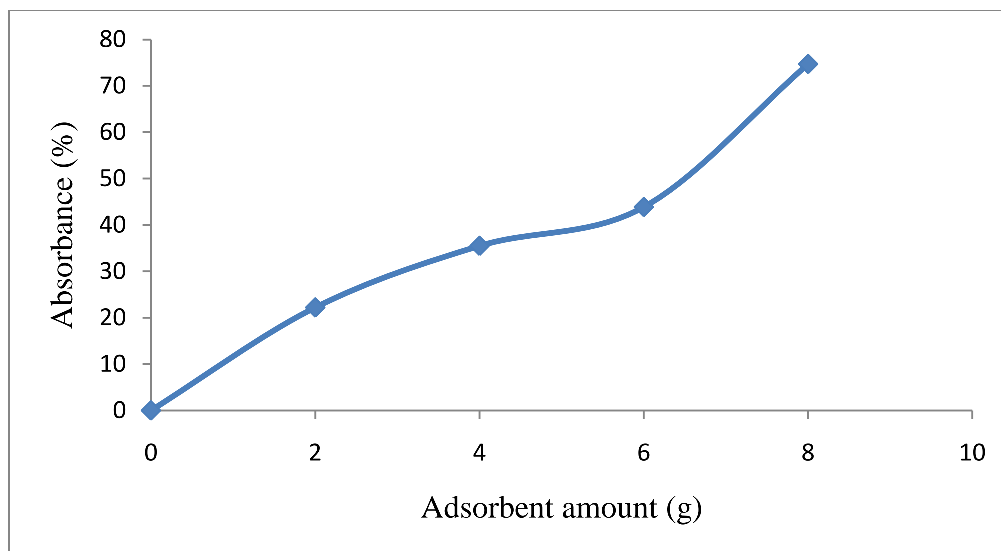

Figure A.1 Effect of adsorbent amount for silica.

Table A.2: Effect of concentration for silica.

Concentration (mg/L)	Before	After	% Absorbance
Blank	0	0	0
100	0.096	0.133	27.8
200	0.035	0.126	72.2
300	0.024	0.127	81.12
400	0.059	0.179	81.76
500	0.026	0.153	83

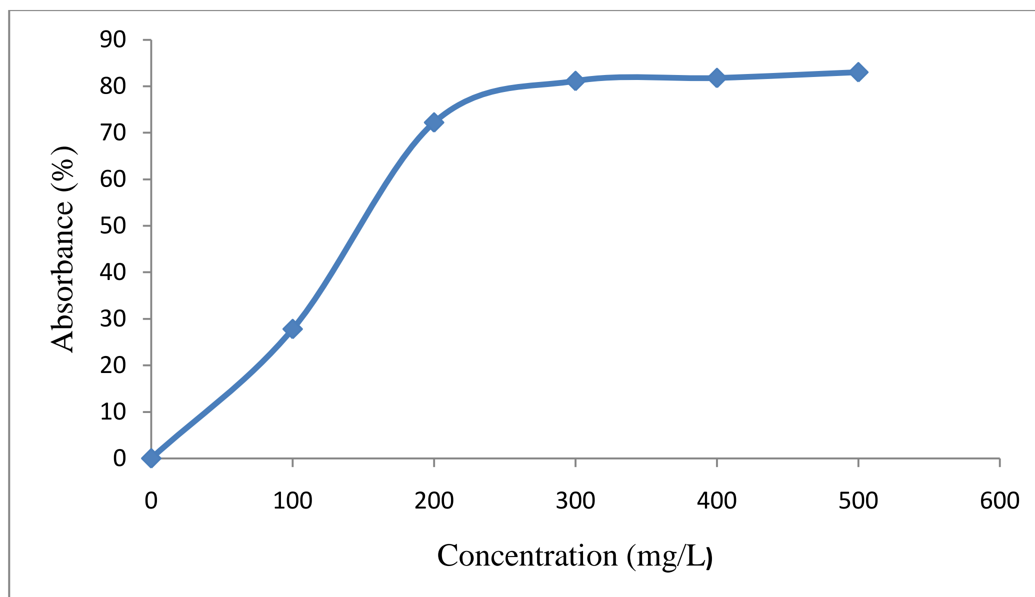

Figure A.2 Effect of concentration for silica.

Table A.3: Effect of adsorbent amount for polymer. (without template)

Adsorbent amount (g)	Before	After	% Absorbance
Blank	0	0	0
2	1.658	2.212	25
4	1.475	2.162	31.8
6	1.696	2.405	29.5
8	1.382	2.14	35

Table A.4: Effect of concentration for polymer (without template)

Concentration (mg/L)	Before	After	% Absorbance
Blank	0	0	0
100	0.239	0.417	42.69
200	0.252	0.596	57.72
300	0.202	0.642	68.54
400	0.262	1.558	83.18
500	0.354	2.155	83.57

Table A.5: Effect of adsorbent amount polymer (with template)

Adsorbent amount (g)	Before	After	% Absorbance
Blank	0	0	0
2	0.318	0.413	23
4	0.357	0.666	46.4
6	0.461	0.934	50.64
8	0.25	1.152	78.3

Table A.6: Effect of concentration for polymer (with template).

Concentration (mg/L)	Before	After	% Absorbance
Blank	0	0	0
100	0.299	0.514	41.83
200	0.21	0.589	64.35
300	0.128	0.802	84
400	0.158	1.124	85.9
500	0.161	1.185	86.41

Table A.7: Effect of adsorbent amount on absorbance capacity for polymer.

Adsorbent amount (g)	Before	After	[S]
Blank	0	0	0
2	303.53	359.41	0.279
4	326.47	508.24	0.454
6	387.65	665.88	0.464
8	263.53	794.12	0.663

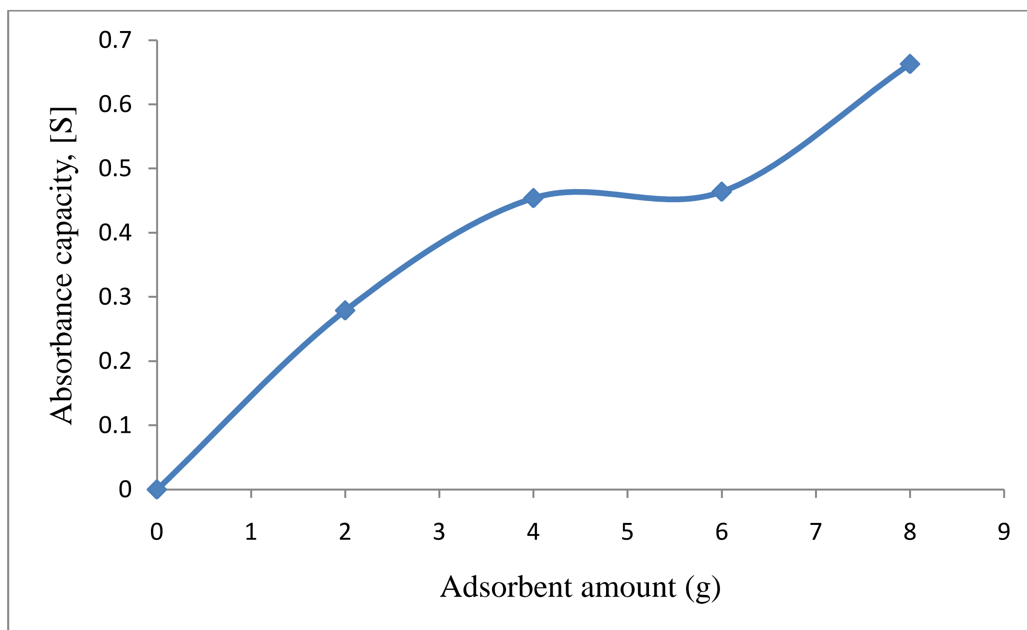

Figure A.3 Effect of adsorbent amount on absorbance capacity for polymer.

Table A.8: Effect of concentration on absorbance capacity for polymer.

Concentration (mg/L)	Before	After	[S]
blank	0	0	0
100	292.35	418.82	0.252
200	240	462.94	0.446
300	191.76	588.24	0.793
400	209.41	777.65	1.136
500	214.12	813.53	1.199

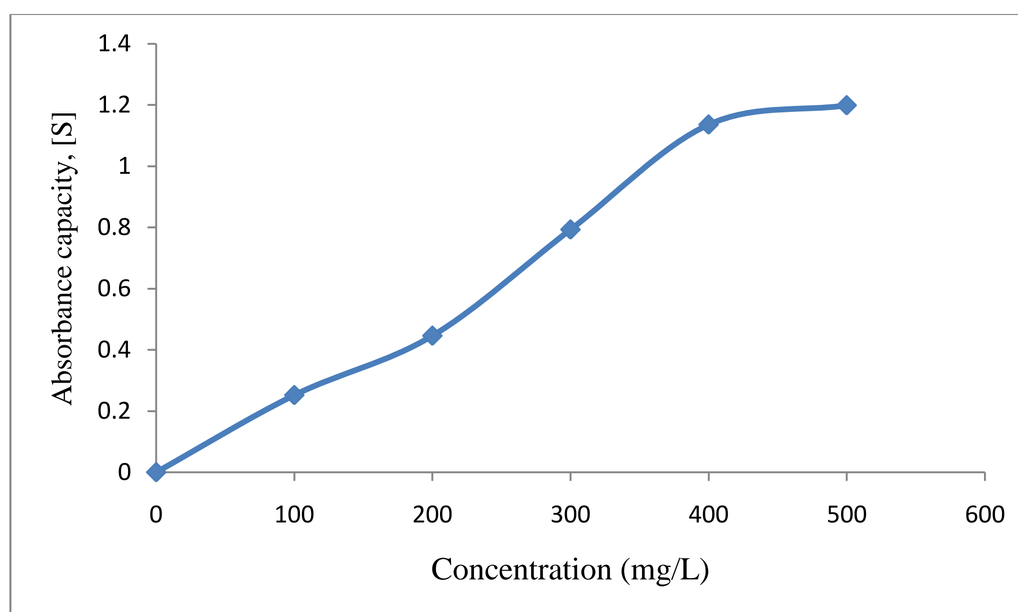

Figure A.4 Effect of concentration on absorbance capacity for polymer.

Table A.9: Effect of adsorbent amount on absorbance capacity for silica.

Adsorbent amount (g)	Before	After	[S]
Blank	0	0	0
2	120.59	121.76	0.0059
4	128.24	134.71	0.016
6	137.29	150	0.021
8	127.65	160.59	0.041

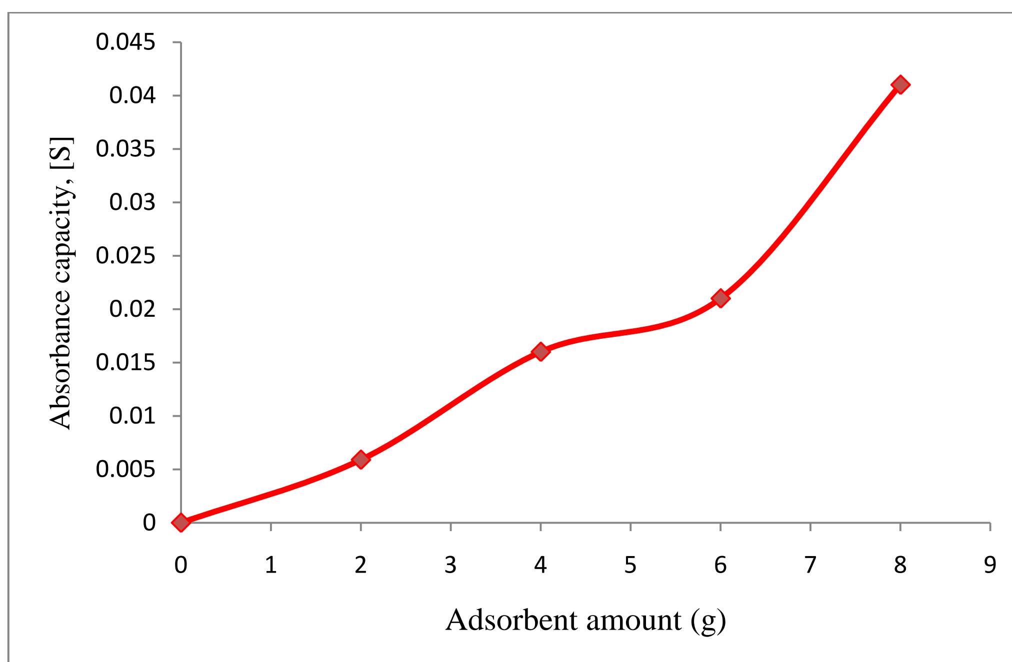

Figure A.5 Effect of adsorbent amount on absorbance capacity for silica.

Table A.10: Effect of concentration on absorbance capacity for silica.

Concentration (mg/L)	Before	After	[S]
Blank	0	0	0
100	172.94	194.71	0.049
200	137.06	190.59	0.107
300	130.59	191.18	0.121
400	151.18	221.76	0.141
500	131.76	206.47	0.149

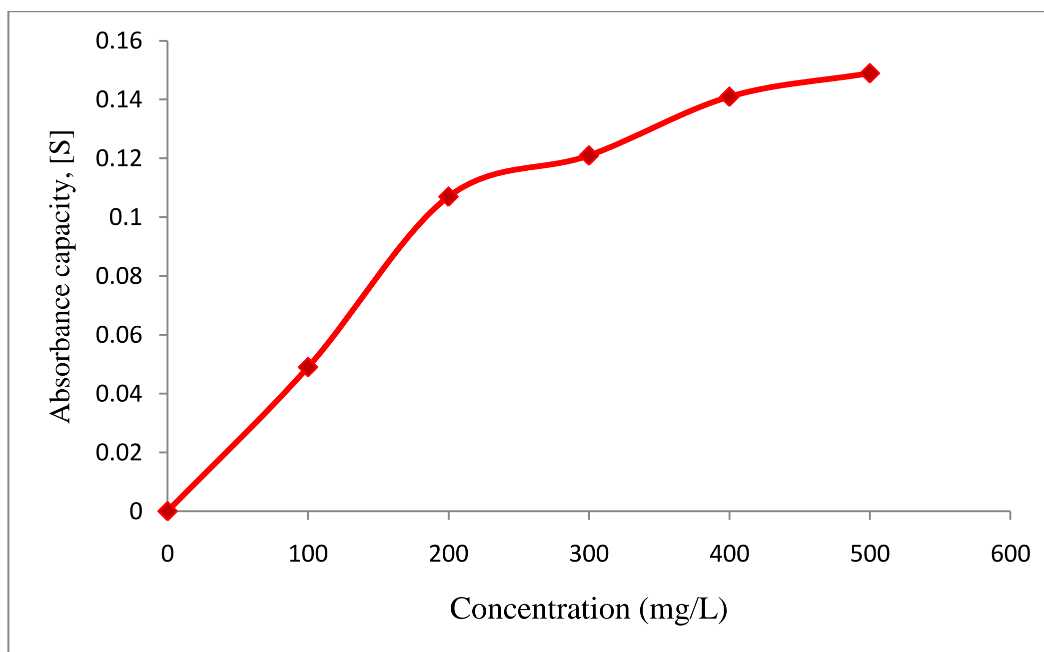

Figure A.6 Effect of concentration on absorbance capacity for silica.

APPENDIX B

Calculation.

1. Calculation for adsorbent amount.

$M_1V_1=M_2V_2$

1000mg/L (V_1) = 100mg/L (0.01L)

V_1 = 1ml phenol solution

Add 1ml phenol solution for each 2g, 4g, 6g, 8g of adsorbent amount.

2. Calculation for concentration.

$M_1V_1=M_2V_2$

1000mg/L (V_1) = 100mg/L (0.01L)

V_1 = 1ml phenol solution

$M_1V_1=M_2V_2$

1000mg/L (V_2) = 100mg/L (0.02L)

V_2 = 2ml phenol solution

$M_1V_1=M_2V_2$

1000mg/L (V_3) = 100mg/L (0.03L)

V_3 = 3ml phenol solution

$M_1V_1=M_2V_2$

1000mg/L (V_4) = 100mg/L (0.04L)

V_4 = 4ml phenol solution

$M_1V_1=M_2V_2$

1000mg/L (V_5) = 100mg/L (0.05L)

V_5 = 5ml phenol solution

3. Hydrolysis calculation.

Molarity = mole/L

= (mole of solute/volume of solution)

For 2M NaOH at 0.06L water;

0.06L solution × 2mole/L NaOH = 0.12 mole NaOH

0.12 mole/ NaOH × 40g/mole NaOH = 4.8 g NaOH

For 2M NaOH, using 4.8g NaOH.

4. Absorbance capacity for polymer of adsorbent amount.

$[S] = \frac{Ci - C_0}{w}$ (v)

At 2g adsorbent;

$[S] = \frac{(359.41 - 303.53)mg/L}{2g} \times 0.01L$

= 0.279 mg/g

At 4g adsorbent;

$$[S] = \frac{(508.24-326.47)mg/L}{4g} \times 0.01L$$

= 0.454 mg/g

At 6g adsorbent;

$$[S] = \frac{(665.88-387.65)mg/L}{6g} \times 0.01L$$

= 0.464 mg/g

At 8g adsorbent;

$$[S] = \frac{(794.12-263.53)mg/L}{8g} \times 0.01L$$

= 0.663 mg/g

5. Absorbance capacity for polymer of concentration.

At 100ml;

$$[S] = \frac{(418.82-292.35)mg/L}{5g} \times 0.01L$$

= 0.252 mg/g

At 200ml;

$$[S] = \frac{(462.82-240)mg/L}{5g} \times 0.01L$$

= 0.446 mg/g

At 300ml;

$$[S] = \frac{(588.24-191.76)mg/L}{5g} \times 0.01L$$

= 0.793 mg/g

At 400ml;

$[S] = \frac{(777.65-209.41)mg/L}{5g} \times 0.01L$

$= 1.136$ mg/g

At 500ml;

$[S] = \frac{(813.53-214.12)mg/L}{5g} \times 0.01L$

$= 1.199$ mg/g

6. Absorbance capacity for silica of adsorbent amount.

$[S] = \frac{Ci-C_0}{w}$ (v)

At 2g adsorbent;

$[S] = \frac{(121.76-120.59)mg/L}{2g} \times 0.01L$

$= 0.0059$ mg/g

At 4g adsorbent;

$[S] = \frac{(134.71-128,24)mg/L}{4g} \times 0.01L$

$= 0.016$ mg/g

At 6g adsorbent;

$[S] = \frac{(150-137.29)mg/L}{6g} \times 0.01L$

$= 0.021$ mg/g

At 8g adsorbent;

$[S] = \frac{(160.59-127.65)mg/L}{8g} \times 0.01L$

$= 0.041$ mg/g

7. Absorbance capacity for silica of concentration.

At 100ml;

$[S] = \frac{(194.71-172.94)mg/L}{5g} \times 0.01L$

$= 0.049$ mg/g

At 200ml;

$[S] = \frac{(190.59-137.06)mg/L}{5g} \times 0.01L$

$= 0.107$ mg/g

At 300ml;

$[S] = \frac{(191.18-130.59)mg/L}{5g} \times 0.01L$

$= 0.121$ mg/g

At 400ml;

$[S] = \frac{(191.18-130.59)mg/L}{5g} \times 0.01L$

$= 0.141$ mg/g

At 500ml;

$[S] = \frac{(206.47-131.76)mg/L}{5g} \times 0.01L$

$= 0.149$ mg/g

APPENDIX C

Standard calibration curve.

Table C.1: Standard calibration curve.

Concentration (mg/L)	Abs 1	Abs 2	Abs 3	Average
100	0.081	0.086	0.085	0.084
200	0.192	0.169	0.180	0.180
300	0.289	0.279	0.282	0.283
400	0.352	0.341	0.353	0.349
500	0.498	0.492	0.495	0.495
600	0.598	0.616	0.583	0.599
700	0.687	0.776	0.781	0.748
800	0.909	0.918	0.922	0.915
900	1.334	1.338	1.335	1.336
1000	1.895	1.918	1.901	1.904

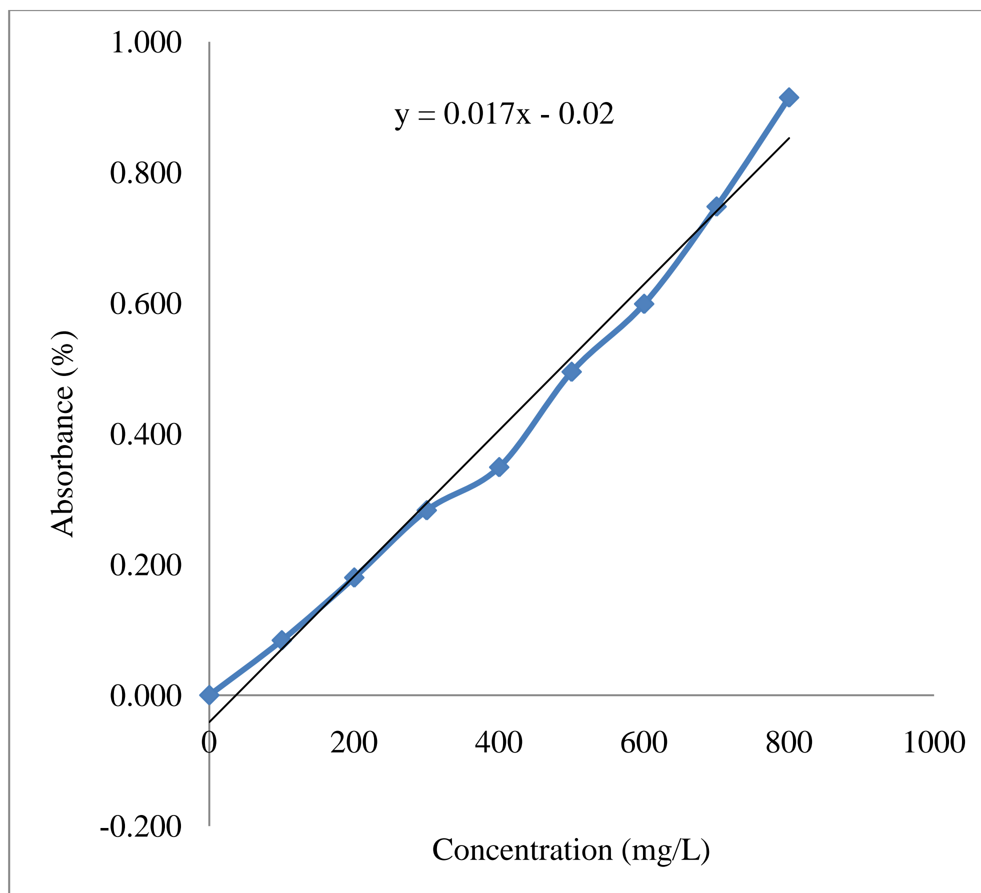

Figure C.1 Standard calibration curve.

APPENDIX D

Figure D.1 Ground polymer with mortar and pestle.

Figure D.2 Sieve tray at 200µm particle size.