SYNTHESIS AND CHARACTERIZATION OF MOLECULAR IMPRINTING POLYMER MICROSPHERES FOR SELECTIVE ADSORPTION OF SYRINGALDEHYDE

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by

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LIST OF ABBREVIATIONS

MIP	Molecules imprinting polymer
SYDM	Syringaldehyde methacrylate
DVB	Divinyl benzene
EGDMA	Ethylene Glycol Dimethacrylate
AFM	Atomic force microscopy
NMR	Nuclear Magnetic Resonance Spectroscopy
FT-IR	Fourier Transform Infrared Spectroscopy
HPLC	High Performance Liquid Chromatography
SEM	Scanning Electron Microscopy
Ra	Average roughness
RMS	Root Mean Square
Rq	Roughness
Rp	Maximum profile peak height
Rv	Maximum profile valley depth
Rz	Ten Point Height

LIST OF SYMBOLS

C^0	Molar concentration before binding
C_t	Molar concentrations after binding
V	Volume
W	Mass
μ_0	Chemical potential in standard state
μ	Chemical potential in actual state
R	Universal gas constant, 8.314 $J^{-1}mol^{-1}K^{-1}$
T	Absolute temperature in Kelvin
Q	Absorbance of MIP microspheres
n_0	Number of moles
ΔH_{ads}	Adsorption enthalpy
ΔS_{ads}	Adsorption entropy
B	Quantity adsorbed by the column
k	Affinity constant
q	Binding site density
n	Heterogeneity parameter
S	Binding amount
C	Celcius

SINTESIS DAN PENCIRIAN POLIMER PERCETAKAN MOLEKUL MIKROSFERA BAGI PENJERAPAN TERPILIH SIRINGALDEHID

ABSTRAK

Polimer percetakan (MIP) berasaskan molekul siringaldehid yang berbentuk mikrosfera telah disintesis menggunakan teknik pempolimeran penyulingan-pemendakan serta ciri fizikal dan kimia, pengecaman spesifik, penjerapan serta sifat pengikatan siringaldehid terhadap MIP telah dikaji. Pada awalnya, monomer bagi polimer percetakan menggunakan siringaldehid metakrilat telah disintesis dan dicirikan menggunakan kaedah FT-IR, ^{13}C-NMR dan ^{1}H-NMR spektroskopi. Seterusnya, monomer tersebut ditindakbalas dengan dua jenis polimer rangkai-silang iaitu etilena glikol dimetakrilat (EGDMA) dan divinil benzena (DVB) dengan menggunakan asetonitril sebagai pelarut serta azobisisobutironitril (AIBN) sebagai penggerak. Pembolehubah yang boleh dikawal seperti tempoh pempolimeran, nisbah polimer rangkai-silang terhadap monomer, jenis polimer rangkai-silang dan isipadu pelarut turut dikaji. Kaedah penganalisaan FT-IR, SEM dan AFM telah dilakukan untuk mengkaji ciri fizikal, kimia dan struktur mikrosfera MIP. Keupayaan pengikatan serta keafinan MIP yang telah disintesis ditentukan menggunakan kaedah HPLC. Keupayaan kimia bagi penjerapan dan keupayaan pengikatan MIP tersebut telah dikaji dengan menggunakan model isoterma penjerapan Freundlich-Langmuir. MIP yang dihasilkan melalui kaedah ini didapati berbentuk mikrosfera dengan saiz 1.4-1.7 μm selepas pempolimeran selama 24 jam. Hasil pempolimeran bagi SDM-2 ialah 80.1% dan bagi SDV-3 ialah 78.6%. Polimer mikrosfera tersebut menunjukkan keafinan dan pemilihan terhadap

siringaldehid apabila diuji bersama dengan campuran analognya, vanilin dan *p*-hidroksibenzaldehid. Kadar nisbah bagi polimer rantai-silang terhadap monomer serta isipadu pelarut mempengaruhi saiz partikel serta kalakuan pengikatan. Penjerapan polimer bukan percetakan (NIP) adalah kurang daripada 5 μmol/g. Sedangkan, penjerapan bagi MIP ialah sebanyak 80% terhadap siringaldehid pada 40 ˚C. Keupayaan kimia bagi penjerapan siringaldehid pada polimer SDM-2 adalah sebanyak -22.69 kJ/mol, yang mana adalah lebih tinggi, dibandingkan dengan vanilin dan *p*-hidroksibenzaldehid.

SYNTHESIS AND CHARACTERIZATION OF MOLECULAR IMPRINTING POLYMER MICROSPHERES FOR SELECTIVE ADSORPTION OF SYRINGALDEHYDE

ABSTRACT

The syringaldehyde based molecularly imprinted polymeric microspheres (MIP) were synthesized using distillation-precipitation polymerization technique, and their physico-chemical nature, specific recognition, adsorption and binding behaviours were investigated in the present thesis. Initially, the monomer for the MIP microspheres (syringaldehyde metacrylate) was synthesized and characterized using Fourier-Transform Infrared (FT-IR), ^{13}C and ^{1}H Nuclear Magnetic Resonance (NMR) technique for compound structural verification and its purity. Subsequently, the monomer was reacted with two different types of cross-linkers: ethylene glycol dimethyacrylate (EGDMA) and divinyl benzene (DVB) using acetonitrile as the solvent and azobisisobutyronitrile (AIBN) as initiator. The controlable variables such as duration of polymerization, ratio of cross-linker to monomer, types of cross-linkers and solvent volume were also investigated. FT-IR, Scanning Electronic Microscope (SEM) and Atomic Force Microscope (AFM) analysis were carried out to study the physico-chemical and structural properties of the MIP spheres. The binding capacity and the affinity of the synthesized MIPs were determined using the HPLC method. The chemical potential for adsorption and binding capacity of the MIP spheres were also investigated using Freundlich-Langmuir adsorption isotherms. The resultant MIP were found to be spherical with the size of 1.4-1.7 μm

after 24 hours of polymerization. The polymer yield obtained for SDM-2 was 80.1% whereas for SDV-3 was 78.6%. These polymeric microspheres exhibited affinity and selectivity towards syringaldehyde when tested with a mixture of its analogues, vanillin and *p*-hydroxybenzaldehyde. The ratio of the cross linkers against monomer as well as volume of solvent greatly influenced the particle size and binding behaviour. The adsorption of non-imprinting polymers was found to be less than 5 µmol/g while the imprinting polymers showed about 80% adsorption at 40 ˚C for syringaldehyde. The chemical potential for adsorption of syringaldehyde is -22.69 kJ/mol, which is very high, in comparison to vanillin and *p*-hydroxybenzaldehyde for the SDM-2 imprinting polymer.

CHAPTER 1

INTRODUCTION

1.1 An overview

The science of shape and size recognition has become a treasured skill, for appearance is an important aspect that identifies an object of its given name such as a car, a banana plant or a tower. However, we need additional tools to recognize a bacteria or a molecule which is too small to be identified by our naked eyes. A widely known fact is that no two molecules are alike. Therefore, many efforts have been carried out in order to selectively recognize and separate a molecule from its environment which consists of a mixture of other molecules typically those synonymous in structure. Interestingly, this process can be attributed to the fabrication of dentures.

Firstly, an impression of a patient's dental layout is created by placing a viscous liquid material on the gums and remaining teeth. This material then gives "a copy" of the patient's gums and teeth once it sets into an elastic "mold". This "mold" is later used to make a denture that perfectly fits the patient's mouth cavity. In a way, this process can be considered as a "macro" imprinting technique. Hence, in order to develop a recognition method for a molecule, we can employ molecular imprinting technique which is rather similar to a certain extent, conceptually.

Molecular imprinting technique was defined by (Alexander et al., 2006) as follows:

The construction of selective ligand in synthetic polymers where a template (atom, ion, molecule, complex or a macromolecular assembly including micro-

organisms) is employed in order to facilitate recognition site formation during the covalent assembly of the bulk phase by a polymerization or polycondensation process with subsequent removal of some or all of the template being necessary for recognition to occur in the spaces vacated by the templating species.

Literally, it means a polymer is shaped and fabricated to meet a target molecule (template) and when the template is removed, a cavity is left in the polymer which corresponds to the template shape and size and bonding properties. If this vacant polymer comes across a molecule exactly like the template molecule, it recognizes this molecule and binds to it momentarily. These synthetic smart materials have become a fascination in the field of chemistry due to its recognition capabilities being at par with those of biological receptors (Vlatakis et al., 1993). Among the numerous advantages of the molecular imprinting systems are their robustness over a wide range of pH and temperature while still being able to retain the recognitive attributes over long term usages (Svenson and Nicholls, 2001). Another exquisite characteristic of MIPs are their advantage over biological receptors whereby they can be used in organic solvents without being denatured. Nevertheless, the main motivation for the research and development of MIPs are definitely the time and cost that can be saved or an environment-safe procedure when compared to current common practices in separation like crystallization, solvent extraction and distillation. The needs for procedures like MIPs is widely justified by the exponential increase in research publications pertaining to this field (Alexander et al., 2006).

1.2 Problem Statement

Syringaldehyde is a precious compound with a myriad of applications ranging from pharmaceuticals to biological control in the ecosystem and it naturally occurs in minute amount. Conventional extraction and purification methods may no longer be relevant for selective separation at μmol level. Therefore, one of the best modern tools developed for selective separation or and quantification would be molecular imprinting especially for substances like syringaldehyde which naturally coexist with several structural analogues. Furthermore, previous study done by Ibrahim et. al, 2008 on the purification on vanillin using molecules imprinting polymer which were fabricated using bulk polymerization. This research serves as a platform for a more uniform and microspheric imprinting polymers and to understand adsorption and selectivity properties of these advanced materials. Syringaldehyde was chosen for this research due to its availability in abundance in the pulping waste of oil palm empty fruit bunch. This research is expected to contribute to the conversion of waste into a value-added compound.

1.3 Research Objectives

The objectives of this research are:

i. To synthesize specific molecular imprinting polymer microspheres for syringaldehyde
ii. To investigate the characteristics of the syringaldehyde imprinting polymer microspheres
iii. To determine the specificity and adsorptivity of syringaldehyde imprinted microparticles towards syringaldehyde in a mixture of phenolic aldehydes.
iv. To determine the capacity of the molecular imprinting polymer microspheres.

1.4 Research Scope

In order to fulfill the objectives mentioned, the research is divided into smaller scopes:-

i. The semi-covalent synthesis process for syringaldehyde specific molecular imprinting polymer is studied in this research in terms of cross-linker to monomer ratio, duration of polymerization and volume of solvent used.

ii. The two common cross-linkers ethylene glycol dimethacrylate, EGDMA and *o*-divinyl benzene, *o*-DVB are used in this synthesis

iii. The characterization of the monomer is done by Fourier Transform Infra-red, FT-IR and Nuclear Magnetic Resonance, NMR non-destructive spectroscopic methods

iv. The characterization of synthesized syringaldehyde imprinting polymers are carried out using Fourier Transform Infra-red spectroscopy, FT-IR, Scanning Electron Microscopy, SEM and Atomic Force Microscopy, AFM

v. The adsorption concentration of syringaldehyde for the imprinting and non-imprinting polymers are measured using High Performance Liquid Chromatography, HPLC.

1.5 The outline of the thesis

This thesis focuses on synthesizing and characterizing molecules imprinting polymer which are more uniformly shaped and with the capability to adsorb the intended target molecule i.e, syringaldehyde. In order to fabricate these imprinting microspheres, common polymerization method called precipitation-distillation is utilized. To complement the synthesis method, a lesser known semi-covalent imprinted polymers approach is explored to achieve the objectives of this study. The properties of the imprinted polymers are studied from the aspect of morphology, adsorption ability and selectivity. A concise literature review is discussed in the following chapter pertaining to this study, followed by materials used and methods of experimentation and the results obtained from the study are analyzed and discussed in Chapter 4. To conclude the research, a brief summary on this research findings and recommendations for future are suggested.

CHAPTER 2

LITERATURE REVIEW

2.1 Molecular Recognition

Molecular recognition has been and is presently receiving a great deal of scientific attention due to its vast array of applications. Molecular recognition in biological contexts can be exemplified by protein-protein interactions, inter-lipid interactions in membranes and mechanism of protein-based catalysis. Taking the protein-based catalysis as an example, Figure 2.1 illustrates how the host molecule or the enzyme recognizes its substrate by shape and conformation. This crucial phenomenon in biological systems has driven modern chemical scientists to mimic such systems to lead the development of new technologies.

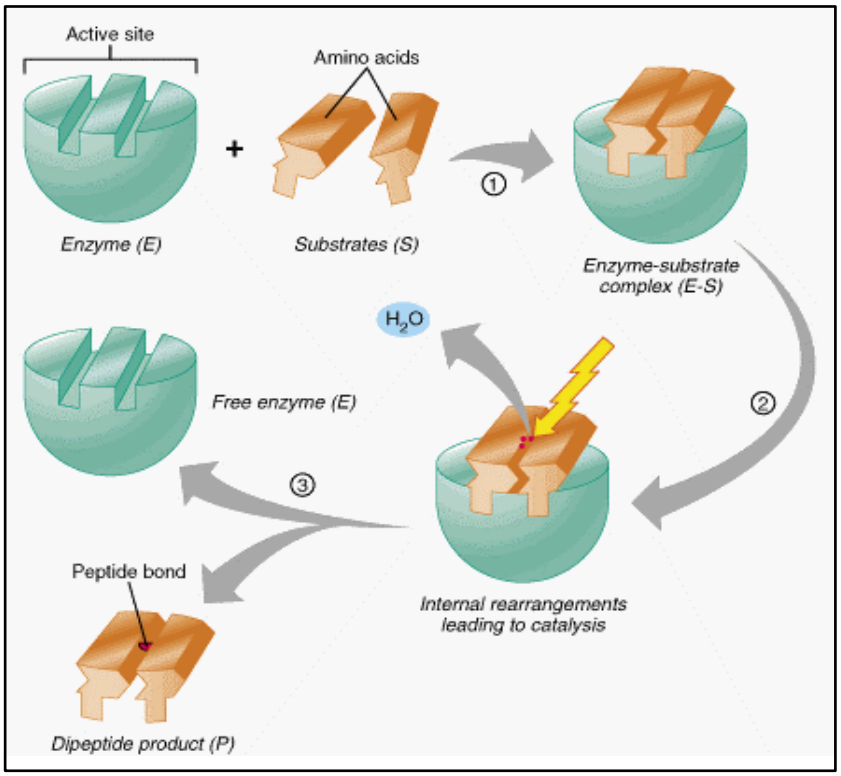

Figure 2.1 Enzyme-substrate conformation in the catalysis of amino acids to form peptides (Campbell et al., 2011).

In this illustration, the amino acids were recognized by the enzyme due to its morphology. The substrate and only this substrate will fit perfectly in this enzyme for further reaction or transformation. This idea became the key enabling technology for modern day separation and sensing materials. Its application at the micro scale has catapulted molecular recognition to a much higher level. Selectivity and molecular recognition using molecularly imprinted materials was discovered by Soviet scientist, M.V. Polyakov in the 1930s who worked on the impression and the effects of the presence of benzene, toluene and xylene during its preparation using silica pore structure (Polyakov et al., 1931). However, it was not until 1972 when Wulff and Klotz reported the preparation of polymers with predetermined ligand selectivity which marked the technology as it is known today (Andersson and Nicholls, 2000). They later coined the term molecularly imprinted polymers (MIP) for their functional polymer. In the past four decades, MIP has become a vast and fast developing field in molecular recognition.

2.2 Molecular Imprinting Polymers

MIPs are smart materials which have a “memory” of the functional groups and precise cavity mimicking the template molecule. The embedded key molecules or templates are then extracted to leave a complementary morphology of the original template molecules. MIPs are designed in such a way to recognize selectively the molecules of interest specifically in the presence of structurally and functionally similar compounds.

Using the enzyme-substrate catalysis as a model representing Fischer’s “Lock-and-Key” concept, the molecular imprinting technology can be explained as follows. The intended molecule for imprinting is classified as “target molecules” or “template molecules” similar to the substrate in the catalysis model. The role of the

enzyme is denoted by the functional monomers in MIPs. The “molecular glues” that bind the substrate to the enzyme in the exemplified model referring to Figure 2.1 are unanimously called cross-linkers in the MIP system. The cavities formed in the polymerization arrangement after the removal of target molecules are called “binding sites”, “receptor sites” or “recognition sites”.

2.2.1 Approaches to synthesis of MIP

Knowing the basic concept of what MIPs consists of; the next step would be in understanding the “how’s” of developing an imprinting polymer using syringaldehyde as target molecules. Before that a brief history on the imprinting methods or approaches are detailed as follows. In general, MIPs can be synthesized using the covalent, non-covalent or the semi-covalent approaches.

Wulff G. and co-researchers introduced the covalent imprinting methods for the first time. These researchers successfully synthesized phenyl-R-D-mannopyranoside imprinted molecules. The polymerization method included 4-vinylphenylboronic acid as the functional monomer and ethylene glycol dimethacrylate (EDMA) as a cross-linker (Wulff and Sarhan, 1972). The template was covalently bonded to the polymer and it had to be cleaved in order for re-binding processes. During the re-binding process, a reformation of the covalent bond between the template and the polymer chain was observed. However, to design an appropriate template-monomer molecule with readily reversible covalent bonds under mild pressures and temperatures can be a rather arduous and un-economical. At present, there are three imprinting approaches: covalent, non-covalent and semi-covalent as shown in Table 2.1.

A non-covalent synthesis method was first experimented by Mosbach K. and team by polymerizing L-phenylalanine anilide (template) and methacrylic acid as the

functional polymer (Mosbach and Kempe, 1995). This pre-arrangement produced a complex which contains hydrogen bonds and electrostatic bonds between the monomer and complex. This complex is copolymerized with ethylene glycol dimethacrylate, a cross-linking agent. The template molecule was extracted from the polymer matrix which leaves behind active binding sites for molecules with exact space and bonding characteristics as the template molecule to selectively rebind. This reversible binding and interactions occurs mainly through hydrogen bonds and even through ionic interactions or other weak intermolecular forces like Van der Waals interactions, π-π interactions and others.

Table 2.1 Approaches to molecular imprinting

Imprinting Approach	**Nature of the Bonds**	
	Template and Monomer	**Analytes and Polymer**
Covalent	Covalent	Covalent
Non-covalent	Non-covalent	Non-covalent
Semi-covalent	Covalent	Non-covalent

As you can see, this method is relatively simpler than the covalent synthesis method since lesser synthetic steps are involved in the template preparation. The versatility of the process which permits a wider range of templates to react with monomers to form the imprinting polymer makes it a better option than covalent synthetic approach. However, the downside of the non-covalent approach is the generation of non-specific or heterogeneous reception sites distributed randomly around the binding sites. As a consequence, undesirable molecules could be attracted towards these sites leading to low purification yield. The low yield of functional high-affinity receptor sites (relative) to amount of template present in the pre-polymerization mixture might have been contributed by the change in morphology after template extraction process.

Therefore, a semi-covalent approach was developed by Whitcombe and co-researchers. The semi-covalent approach comprises of two main methodologies. The first method is where the template and monomer is linked directly and another which uses a spacer or a cross-linker to connect them. In the idea proposed by (Whitcombe and Vulfson, 2001), a carbonate ester was used to covalently bound the template to the monomer which when hydrolyzed will release CO_2. A ester (COO) link functions as a sacrificial spacer to link the cholesterol template to a phenolic residue in the polymer matrix. The theoretical framework for semi-covalent polymerization technique is given in Figure 2.2.

Semi-covalent imprinting method has surpassed the impediments of homogenous binding sites, reduced non-specific sites generation and possible reduced redundant monomers in the imprinting polymer synthesized using the covalent and non-covalent method. However, there still exist certain limitations to this approach which is the complete extraction of templates from the polymer matrix.

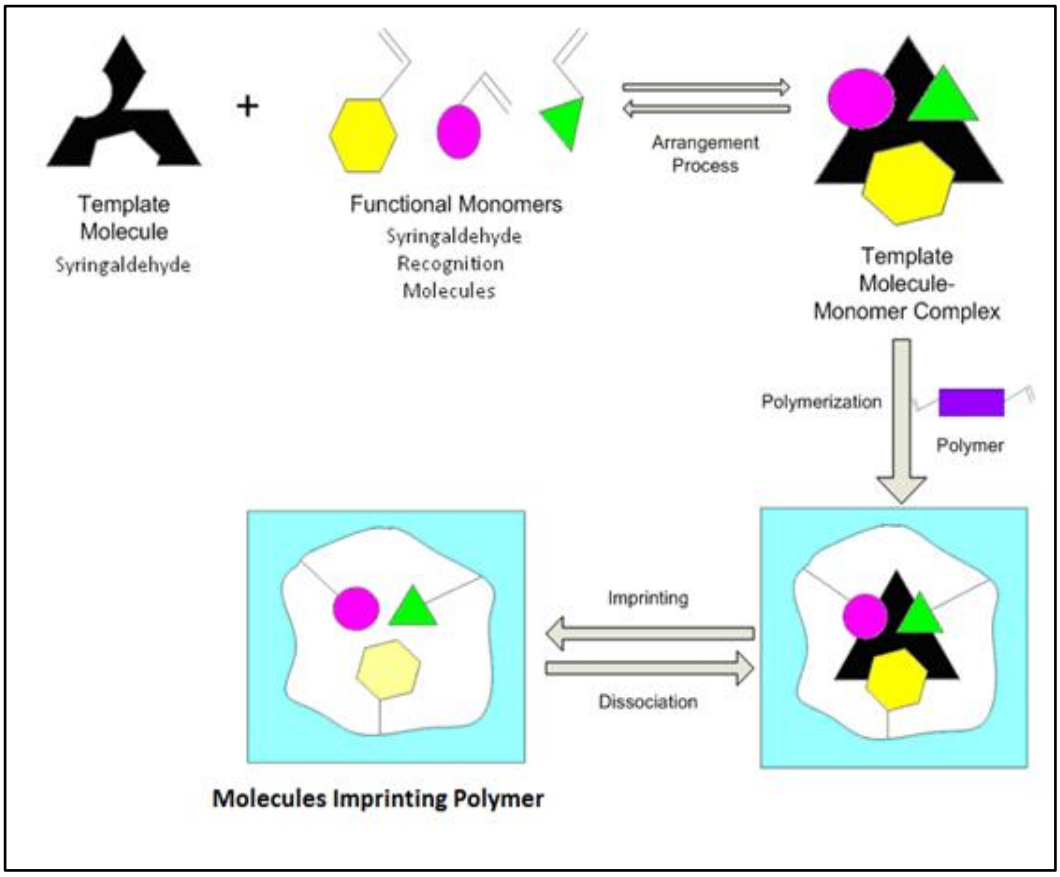

Figure 2.2. Theoretical framework of semi-covalent-type imprinting technique (Ibrahim et al., 2009)

2.2.2 A summary on MIP synthesis strategies

Using the covalent, non-covalent and semi-covalent imprinting approaches, the morphology and shape of the MIP can be controlled. The outcome of the MIP polymerization process such as beads, films and gels largely depends on the polymerization methods chosen. Choosing the right method and fine-tuning the procedures can yield a product of functional capability. A summary of MIP synthesis strategies are summarized in Table 2.2.

Traditionally, MIPs were polymerized by using cross-linkers which produced monolithic bulk polymers which had to be grinded (Ibrahim et al., 2009). The

grinded polymer particles had very irregular shapes and sizes. Once grounded into powder, there is a high likeliness for the active binding sites to be cleaved and lost.

Table 2.2 A comparison on various imprinting polymer synthesis techniques

Polymerization Methods	Procedure & Mechanism	Product	Advantages	Disadvantages	References
Bulk	Monomer, cross-linker, initiator and solvent are polymerized and ground into fine powder.	Irregular particles in shape and size	Easy. Free from surfactant.	Low surface area. Poor purity. Poor yield of active sites.	(Che Ku et al., 2008) (Takeda and Kobayashi, 2005a)
Emulsion	Surfactants are added to stabilize monomer micelles in a non-solvent media. Once polymerization is complete, evenly dispersed polymer beads are formed.	Spherical beads obtained, typically< 1µm	Uniform, controllable size, micro to nano scale beads.	Interference by surfactants. Poor purity. Poor yield.	(Dvorakova et al., 2010)
Precipitation	Monomers and other components are dissolved in a desired media. The polymer precipitates to form spherical particulate.	Spherical beads 1µm<id<10 µm	Fairly simple. No surfactants. High purity.	The polymer beads obtained are not necessarily uniform. Poor yield.	(Wang et al., 2011)
Suspension/ Dispersion	Droplets of monomer, initiator and solvent are dispersed in a continuous phase is maintained by continuous mechanical stirring whereby usage of surfactant is optional. The polymer beads are formed from these droplets once polymerization is completed.	Spherical beads 20µm<id<200 µm	Fairly simple. Good yield. High purity.	Interferences by surfactants (if used). Particle size is in a wide range.	(Son et al., 2011)

Table 2.2 A comparison on various imprinting polymer synthesis techniques

Polymerization Methods	Procedure & Mechanism	Product	Advantages	Disadvantages	References
Multi-step swelling	Seeds are produced using emulsion polymerization technique and made to swell in an activating solvent. The monomers, cross-linkers and other components are dispersed in the solvent. The resultant polymers are droplet-like beads absorbed on the swollen seeds surface.	Spherical beads 1μm<id<20μm	Excellent packing for HPLC column. Good yield. High purity. Very effective.	Complex process and expensive.	(Hoshina et al., 2011, Yang et al., 2010)
Imprinting on pre-formed beads	MIP layer is adsorbed on the surface of pre-formed particles by modifying or grafting method to produce composite beads.	Depends on the pre-formed particles size	High separation capacity. Special functions. Good yield. High purity. Very effective.	Complex. High Cost.	(Yang et al., 2010)

2.3 Syringaldehyde as target molecule

Over the years, however, a meager number of articles dedicated to syringaldehyde have been published. This can probably be attributed to its simple synthesizability and the lack of understanding of its true capacities. The molecular structure of syringaldehyde is given in Figure 2.3 and its physical properties are provided in Table 2.3.

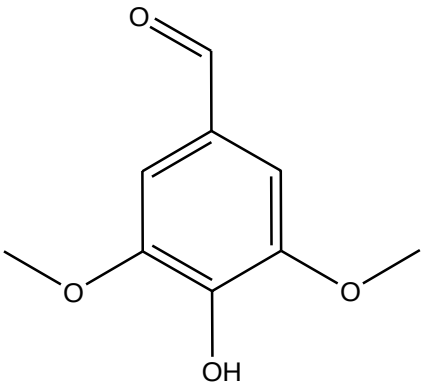

Figure 2.3 Structure of syringaldehyde

Table 2.3 Physical Properties of Syringaldehyde (Ibrahim et al., 2012)

Property	**Data**
Molecular Formula	$C_9H_{10}O_4$
CAS number	134-96-3
Molecular Weight	182.18
Physical State	Solid (yellow-brown)
Melting Point	110-113 ˚C
Boiling Point	192-193 ˚C
Water Solubility	Insoluble

2.4 Properties of syringaldehyde

Advancements in analytical instruments coupled with breakthroughs in chemistry and pharmacology have allowed for the identification, quantification, and isolation of phenolic aldehydes for the diverse applications such as antioxidants, antifungal or antimicrobial, and anti-tumorigenesis agents in pharmaceuticals. Syringaldehyde is vastly used as a precursor for pharmaceutical antifungal products like Trimethoprim and Bactrim (Tripathi et. al). The structures of Trimethoprim and Bactrim are given in Figure 2.4. In the food industry there is also a tendency to utilize naturally occurring flavor compounds that exhibit antioxidant and antimicrobial properties, hence providing a potential source of non-synthetic preservatives and additives. Only preliminary in vitro tests have been reported in most cases, but a new potential research area and application of syringaldehyde has been identified. Keeping this in mind, some of the reported properties of syringaldehyde are exemplified in Tables 2.4 and 2.5.

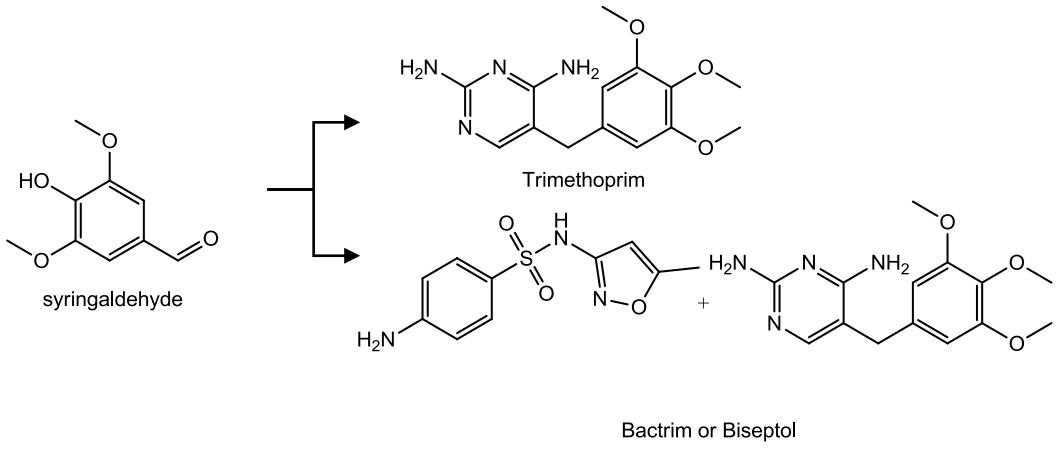

Figure 2.4 The structures of syringaldehyde, as well as Trimethoprim, Bactrim, or Biseptol (Ibrahim et al., 2012)

Table 2.4 A summary of bioactive properties of syringaldehye

	Bioactive Properties	Description	References
1.	Antioxidant capacity	• Wines aged in oak barrels exhibits increased levels of antioxidants due to the presence of phenolic aldehydes like syringaldehyde. • Phenolic extracts containing syringaldehyde from wild rice hulls and olive tree pruning. • Syringaldehyde shows high antioxidant capacity when tested using Trolox C and DPPH method. • The dimethoxy substitution in syringaldehyde as well as its syringol moiety was acknowledged for exhibiting enhanced antioxidant properties. Test conducted using Trolox and CB assays. • Syringaldehyde-based dendrimers exerted strong antioxidant character-istics but reduced pro-oxidant (mutagenicity and carcinogenicity on healthy cells) effects.	(Canas et al., 2008) (Matejícek et al., 2005) (Asamarai et al., 1996), (Bortolomeazzi et al., 2007) (Boundagidou et al., 2010) (Cao et al., 1997) (Lee et al., 2009)
2.	Antimicrobial/ antifungal activity	• Promising effect against *Candida guilliermondii* • Tested against strains of *Plasmodium falsiparum* and showed a mild anti-malarial activity with an IC_{50} value of 21.4 ± 8.2 µg mL^{-1} • Reduced the population of *Staphylococcus aureus* (Gram +), *Klebsiella pneumonia* (Gram -), and *Pseudomonas aeruginosa* (Gram -) by 70% using the ASTM Standard Test Method E2149-01 on paper and pulp.	(Gurpilhares et al., 2006, Kelly et al., 2008, Cheplogoi et al., 2008, Fillat et al., 2012)
3.	Anti-oncogenic property	• Syringaldehyde was noted to show potency in inhibiting the formation of OPBA and NNAL (lung oncogenesis metabolites) in hepatic microsomes. • Syringaldehyde synergetically with seven other compounds showed a high potential in inhibiting the proliferation of HCT-116 cancer cell lines.	Morse *et al.* (1995) (González-Sarrías et al., 2012)

Table 2.5 A summary of other properties of syringaldehye

	Other Properties	Description	References
1.	Mediator	• First natural mediators in the degradation of indigo carmine by bacterial laccase • A redox mediator for recalcirant dye de-colorization • Syringaldehyde shows high antioxidant capacity when tested using Trolox C and DPPH method. • Bio bleaching and delignification of pulp and paper. • A mediator with both oxidoreductive enzymes and inorganic catalysts for the conversion of cyprodinil	(Kawai et al., 1989), (Singh et al., 2007) (Satar and Husain, 2009) Camarero *et al.* (2005), (Camarero et al., 2007) (Murugesan et al., 2009) (Moldes et al., 2008) (Kang et al., 2002), Dec et al., 2004
2.	Inhibitors for hydrolysis	• Inhibition of xylose conversion into xylitol by *Gluconobacter oxydans* • Tested against strains of *Plasmodium falsiparum* and showed a mild anti-malarial activity with an IC_{50} value of 21.4 ± 8.2 µg mL^{-1} • Reduced the population of *Staphylococcus aureus* (Gram +), *Klebsiella pneumonia* (Gram -), and *Pseudomonas aeruginosa* (Gram -) by 70% using the ASTM Standard Test Method E2149-01 on paper and pulp. • Selective inhibition of urease instead of α-chymotrypsin that prevents peptic ulcers.	(Zaldivar et al., 1999) (Buchert and Niemela, 1990), (Delgenes et al., 1996, Taherzadeh et al., 1999, Klinke et al., 2004, Klinke et al., 2001)
3.	Organic markers in wood smoke	• Used as a molecular marker for biomass smoke to monitor global climate • Syringaldehyde synergetically with seven other compounds showed a high potential in inhibiting the proliferation of HCT-116 cancer cell lines.	(Simoneit et al., 1995), (Robinson et al., 2006), (Simoneit, 2002)
4.	Biological activity	• *Agrobacterium tumefaciens* virulence gene inducer that causes death of *Acanthoscelides obtectus* beetles that destroys crops.	(Delmotte et al., 1991, Lee et al., 1996), (Regnault-Roger et al., 2004)

2.5. Polymerization process of the MIPs

The molecular imprinting system reported in the present thesis is a semi-covalent system which is less explored. In general, the semi-covalent polymerization process comprises of a monomer, cross-linker (*o*-DVB and EGDMA), template molecule (syringaldehyde), initiator (AIBN) and a porogenic solvent. The template molecule is similar in geometry and analogous in structure to vanillin and *p*-hydroxybenzaldehyde. Figure 2.5 illustrates the structural similarity of *p*-hydroxybenzaldehyde, vaniline and syringaldehyde respectively. The MIPs exhibits high affinity and selectivity towards the family of related compounds due to similar functional group and structural make-up. Thus, both MIPs and NIPs (non-imprinting polymer) are synthesized to determine the selectivity of the MIP towards syringaldehyde as well as its analogues. In order to assess the selectivity of MIPs towards syringaldehyde, reference polymers or blanks are synthesized without involving any template molecules which are called NIPs.

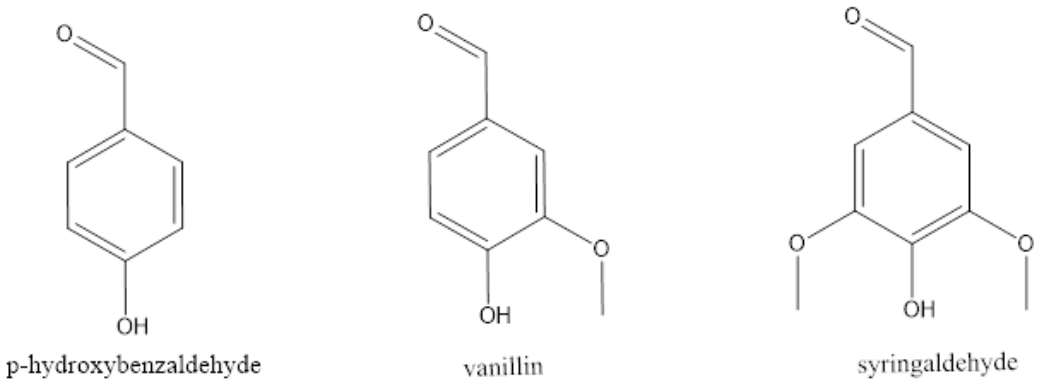

Figure 2.5 An illustration of the molecules analogous to syringaldehyde (Ibrahim et al., 2009)

Optimum processing parameters for the synthesis of MIP for syringaldehyde were investigated with different factors which are most likely to impact the

selectivity and reusability of the MIP towards syringaldehyde. For this purpose, the purity of all starting materials are ensured through recrystallization and only HPLC grade acetonitrile is utilized for synthesis and rinsing. Deoxygenated nitrogen gas was used to purge off oxygen from the pre-polymerization solution. Besides factors like polymerization technique, duration of polymerization, ratio of monomer to cross-linker and volume of solvent used are examined as well. Two types of cross-linkers were used in order to understand the influence of bonding types on the batch-binding properties of imprinted polymers.

In the semi-covalent polymers, *o*-DVB and EGDMA were used as cross-linker and their fraction was high in the polymer. In many other protocols, both *o*-DVB and EGDMA had been successfully employed in non-covalent synthesis methods (Takeda and Kobayashi, 2005b). The cross-linker, *o*-DVB is styrenic and hydrophobic in nature (Cormack and Elorza, 2004) which is copolymerized with syringaldehyde methacrylate to produce a narrow disperse microspheres with active carboxylic acid groups. In order to synthesize this semi-covalent polymer, syringaldehyde was reacted with methacryloyl chloride to form a methacrylate in the first stage. Subsequently, the monomer, SYDM was co-polymerized with *o*-DVB to form the MIP-template network. Syringaldehyde methacrylate is more likely to encourage the formation of inter-chain hydrogen bonding between the carboxylic acid groups to reduce the polymer-solvent interactions during the distillation precipitation copolymerization (Wang et al., 2007). Upon hydrolysis, the template molecule is released and an imprinting cavity with active sites is available for the entrapment of target syringaldehyde molecules. Such method is exclusive for the semi-covalent imprinting technique.

From previous studies (Quesada-Molina et al., 2012, Valero-Navarro et al., 2009) the most common method of synthesizing MIP is by adding the template molecule to an independent monomer, cross-linker and an initiator in a solvent media. The shortcome of this method is that the mechanism by which all these inputs actually form the MIP of interest remain a little vague. However, this shortcome is addressed by the semi-covalent imprinting method by synthesizing a monomer which contains the template molecule. In addition, the template molecule can be easily removed through hydrolysis for the semi-covalent method (Takeda and Kobayashi, 2005b, Poma et al., 2010) The reaction outline of copolymerization of SYDM-co-DVB and its hydrolysis process is illustrated in Figure 2.6 and Figure 2.7.

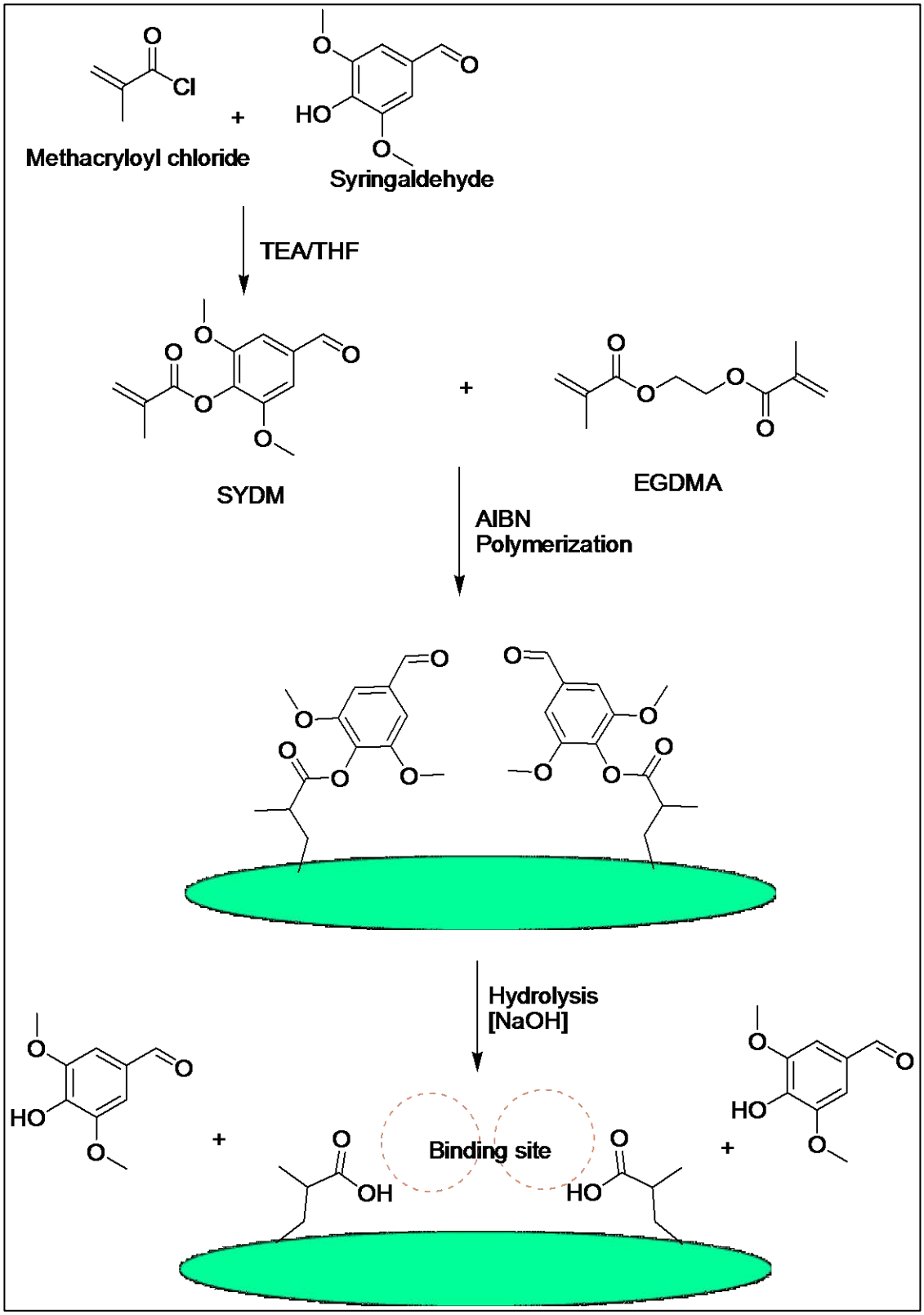

Figure 2.6 An illustration depicting the formation of syringaldehyde binding site

Acetonitrile was chosen as the porogenic solvent because it solubilizes the template even in high concentration. Furthermore, using neat acetonitrile aids the formation of less distorted and more spherically shaped microspheres which will produce uniformed distribution regardless of a styrenic or vinylic cross-linker usage (Son et al., 2011). It exhibited a rather good thermal stability during batch-binding reactions. Nevertheless, the solvent chosen should soluble the templet but it should not dissolve the resulting polymer. Therefore, acetonitrile is the best solvent for this purpose.

A mono-vinyl cross-linker, EGDMA was also selected to synthesize imprinting polymer microspheres. The relatively low viscosity of EGDMA enables it to homogenize the monomer mixture prior to polymerization (Muhammad et al., 2012). Meanwhile, the methacrylate functional group was used in an attempt to allow formation of H-bonds between the template molecule (syringaldehyde) and polymer matrix. The SYDM (monomers) are hydrophilic in nature thus expected to promote inter-chain hydrogen bond formation among the carboxylic acid groups. These inter-chain H-bonding in return reduced the solvent-monomer interactions and increased the interactions of the monomer with EGDMA or *o*-DVB during distillation process. A similar observation was reported by (Bai et al., 2006). The structures of *o*-DVB and EGDMA are given in Figure 2.8.

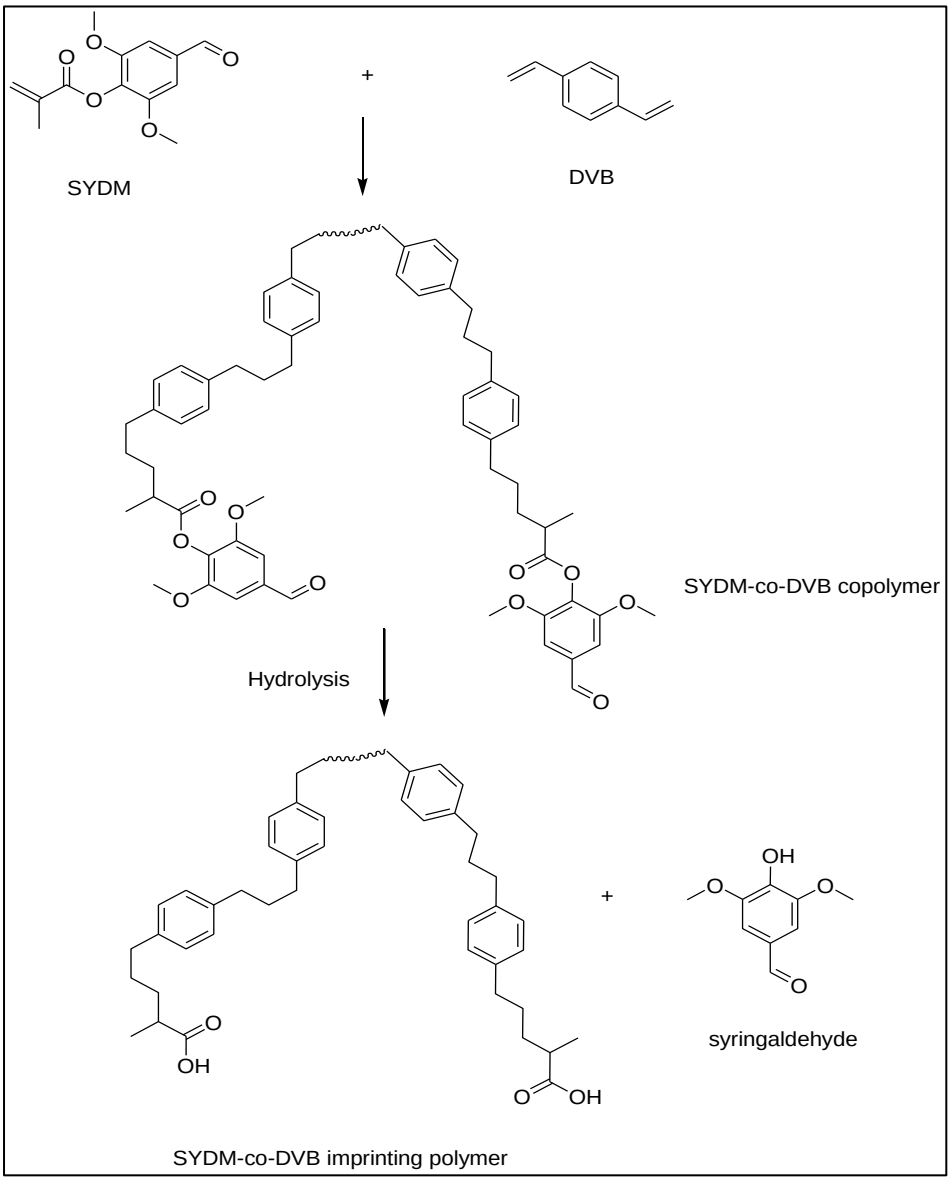

Figure 2.7 Synthesis and hydrolysis of SYDM-co-DVB co-polymer

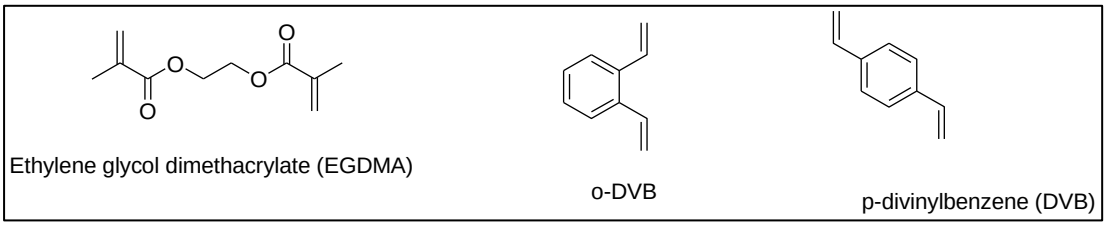

Figure 2.8 Chemical structures of EGDMA and *o*-DVB