DEVELOPMENT OF NANO-SIZED β-CYCLODEXTRIN POLYMERS AS AN ADSORBENT FOR THE EFFECTIVE REMOVAL OF PHENOLIC COMPOUNDS FROM AQUEOUS SAMPLES

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LIST OF ABBREVIATIONS

BPA	Bisphenol A
BPA-MAA-βCD	Bisphenol A inclusion complex
2,4-DCP	2,4-dichlorophenol
2,4-DNP	2,4-dinitrophenol
AC	Activated charcoal
ATR	Attenuated total reflectance
β-CD	Beta-cyclodextrin
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
EPA	Environmental Protection Agency
EU	European Union
Fe_3O_4	Magnetic nanoparticles
Fe_3O_4@ MAA- βCD	Magnetic nanoparticles of MAA- βCD
FTIR	Fourier transform infrared spectroscopy.
JCPDS	Joint Committee on Powder Diffraction Standards
MAA	Methacrylic acid
MAA- βCD	Methacrylic acid functionalized β-cyclodextrin
MIP	Molecularly imprinted polymer
MMIP MAA- βCD	Magnetic molecularly imprinted polymer based on MAA- βCD.
MMIP MAA	Magnetic molecularly imprinted polymer based on MAA.
MNIP MAA	Magnetic non-molecularly imprinted polymer based on MAA.
MNIP MAA- βCD	Magnetic non-molecularly imprinted polymer based on MAA- βCD.

Ms	Magnetic saturation
NIP	Non molecularly imprinted polymer
ppm	part per million
RSD	Relative standard deviation
SD	Standard deviation
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TDI	Toluene-2,4- diisocyanate
TGA	Thermogravimetric analysis
USEPA	US Environmental Protection Agency
VSM	Vibrating sample magnetometer
UV-Vis	Ultraviolet-visible
XRD	X-ray diffractometer

LIST OF SYMBOLS

pKa	Acid dissociation constant
q_e	Adsorption capacity (mg/g)
q_e cal	Calculated adsorption capacity (mg/g)
δ	Chemical shift (ppm)
Cl^-	Chloro group
R^2	Coefficient of determination
ºC	Degree Celsius
Δ	Delta
RL	Dimensional separation factor
Kd	Distribution coefficients
C_e	Equilibrium concentration of adsorbate (mg/L)
emu	Electromagnetic unit
ΔHº	Enthalpy
ΔSº	Entropy
q_e exp	Experimental adsorption capacity (mg/g)
g	Gram
Cf	Final concentration of adsorbate (mg/L)
KF	Freundlich constant
ΔG	Gibb's free energy
R	Gas constant (J/Kmol)
O-H	Hydroxyl group
1/n	Heterogeneity Factor
Co	Initial concentration of adsorbate (mg/L)
J	Joules
K	Kelvin

kJ	Kilojoules
L	Liter
q_m	Maximum adsorption capacity
mg	Milligram
mΩ	Milliohm
N	Number of data points
nm	Nanometer
^{1}HNMR	Proton NMR
Δq (%)	Normalized standard deviation
NO^{2-}	Nitro group
θ	Theta
C	Thickness of boundary layer
V	Volume
λmax	Wavelength of maximum absorbance

PEMBANGUNAN POLIMER β- SIKLODEKSTRIN BERSAIZ NANO SEBAGAI PENJERAP BAGI PENYINGKIRAN BERKESAN SEBATIAN FENOL DARIPADA SAMPEL AKUEUS

ABSTRAK

Penggunaan bahan berasaskan siklodekstrin sebagai penjerap untuk menghilangkan bahan cemar yang berbeza adalah penyelidikan canggih yang menarik perhatian kebanyakan penyelidik di seluruh dunia. Dalam penyelidikan ini, magnetit metakrilik berfungsi-β-siklodekstrin (Fe_3O_4 @ MAA-βCD), polimer molekul bercetak magnetik berdasarkan asid metakrilik berfungsi asid-metakrilik (MMIP MAA-βCD), polimer bukan molekul magnetik berasaskan asid metakrilik berfungsi β- siklodekstrin (MNIP MAA-βCD) polimer bercetak molekul magnetik berdasarkan asid metakrilik (MMIP MAA) dan polimer tidak bercetak magnetik berdasarkan asid metakrilik (MNIP MAA) berjaya disintesis untuk penyingkiran 2,4-diklorofenol (2,4-DNP), 2,4-dinitrofenol (2,4-DNP) dan bisfenol A (BPA) daripada medium akues. Penjerap dicirikan dengan spektroskopi inframerah transformasi Fourier (FTIR), imbasan mikroskopi elektron (SEM), mikroskopi transmisi elektron (TEM), magnetometer getar sampel (VSM), Brunauer-Emmett-Teller (BET), penganalisis termogravimetrik (TGA) dan pembelauan sinar X (XRD). Spektroskopi ^{1}H resonans magnet nukleus (^{1}H NMR) digunakan untuk mencirikan MAA-βCD dan kompleks BPA terangkum-MAA-βCD. Beberapa pembolehubah yang mempengaruhi kecekapan penjerapan sebatian fenolik terpilih telah dianalisis secara mendalam. Fe_3O_4 @ MAA-βCD memberikan jumlah penyingkiran optimum sebatian fenolik telah dikaji pada pH 2 untuk 2,4-DNP, pH 7 untuk 2,4-DCP, dan pH 6 untuk BPA. Sementara itu, masa, kepekatan awal, dos penjerap telah ditetapkan masing-masing

pada 60 min, 10 mg/L, dan 20 mg. Kecekapan penyingkiran optimum pada 60 min adalah 89%, 78%, dan 69% untuk 2,4-DNP, 2,4- DCP dan BPA. Pada masa yang sama, MMIP MAA-βCD bagi penyingkiran BPA memberi masa penjerapan optimum pada 60 min, pH 8, kepekatan analit 10 mg/L, dos penjerap 20 mg dan laju pengadukan 250 rpm. Pada keadaan optimum, kecekapan penyingkiran BPA didapati 96%. MMIP MAA-βCD menunjukkan selektiviti yang tinggi, keafinan dan tindak balas kinetik yang cepat untuk penyerapan BPA berbanding dengan MMIP MAA (tanpa βCD) dan ia dipilih sebagai penjerap yang terbaik. Model urutan pseudo kedua memberikan suaian terbaik untuk hasil kinetik bagi semua penjerap yang dikaji. Model Freundlich dan model Halsey merupakan model-model yang paling sesuai untuk data keseimbangan penjerapan dengan R^2> 0.9563, menunjukkan MMIP MAA-βCD dan Fe_3O_4@MAA-βCD adalah sistem yang heterogen. Eksperimen termodinamik telah membuktikan bahawa sistem penjerapan boleh dilaksana secara termodinamik, eksotermik dan spontan. Hasil menunjukkan bahawa jenis interaksi utama yang terlibat dalam analisis ini adalah ikatan hidrogen, pembentukan kompleks terangkum dan interaksi $\pi - \pi$. Bahan baharu ini merangkumi kedua-dua faedah penggunaan interaksi tuan rumah-tetamu supra magnetik dan sifat pengenalan molekul oleh molekul polimer tercetak. Bahan-bahan yang disintesis berwarna hijau, mudah disediakan, cekap dan mudah digunakan dalam operasi berskala besar tanpa menimbulkan kesan terhadap alam sekitar atau kesihatan. Selain itu, bahan-bahan ini terbukti sebagai penjerap yang berpotensi untuk diguna dalam proses penyingkiran fenolik daripada sampel akues alam sekitar secara rutin.

DEVELOPMENT OF NANO-SIZED β-CYCLODEXTRIN POLYMERS AS AN ADSORBENT FOR THE EFFECTIVE REMOVAL OF PHENOLIC COMPOUNDS FROM AQUEOUS SAMPLES

ABSTRACT

The use of cyclodextrin-based materials as adsorbents for removing different contaminants is cutting-edge research that has caught the attention of many researchers worldwide. In this research, magnetite methacrylic acid-functionalized β-cyclodextrin (Fe_3O_4@MAA-βCD), magnetic molecularly imprinted polymer based on methacrylic acid-functionalized β-cyclodextrin (MMIP MAA-βCD), magnetic non-molecularly imprinted polymer based on methacrylic acid-functionalized β-cyclodextrin (MNIP MAA-βCD, magnetic molecularly imprinted polymer based on methacrylic acid (MMIP MAA) and magnetic non-molecularly imprinted polymer based on methacrylic acid (MNIP MAA) were successfully synthesized for the removal of 2,4-dichlorophenol (2,4-DNP), 2,4-dinitrophenol (2,4-DNP) and bisphenol A (BPA) from aqueous media. The adsorbents were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), transmission electron microscopy (TEM), vibrating sample magnetometer (VSM), Brunauer-Emmett-Teller (BET), thermogravimetric analyzer (TGA) and X-ray Diffraction (XRD). ^{1}H NMR spectroscopy was used to characterize the MAA-βCD and BPA-MAA-βCD inclusion complex. Several variables influencing the adsorption efficiency of the selected phenolic compound have been analyzed in depth. Fe_3O_4@MAA-βCD gave the optimal removal amount of the studied phenolic compounds studied at pH 2 for 2,4-DNP, pH 7 for 2,4-DCP, and pH 6 for BPA. While the contact time, initial concentration, the adsorbent dose was set at 60 min, 10 mg/L, and 20 mg, respectively. The optimum

removal efficiency at 60 min was 89%, 78%, and 69% for 2,4-DNP, 2,4- DCP and BPA. Similarly, MMIP MAA-βCD for removing BPA gave an optimum adsorption time of 60 min at pH 8, 10 mg/L analyte concentration, 20 mg adsorbent dose and 250 rpm stirring speed. At the optimized conditions, the removal efficiency of BPA was found to be 96%. MMIP MAA-βCD showed high selectivity, affinity and fast kinetic response for BPA adsorption compared to MMIP MAA (without βCD) and it was selected as the best adsorbent. The Pseudo-second-order model provided the best fit for the kinetic results in all the studied adsorbents. The Freundlich and Halsey model best fitted the adsorption equilibrium data with $R^2 > 0.9563$, indicating MMIP MAA-βCD and Fe_3O_4@MAA-βCD are heterogeneous systems. Thermodynamic experiments have demonstrated that the adsorption system is thermodynamically feasible, exothermic and spontaneous. The result also revealed that the primary type of interactions involved in this analysis was hydrogen bonding, inclusion complex formation and π–π interaction. These new materials incorporate both the benefit of using supra magnetic host-guest interaction and the molecularly imprinted polymer's molecular recognition properties. The synthesized materials are green, easy to prepare, efficient and easily used in large-scale operations without raising environmental or health concerns. Also, these materials proved to be promising adsorbents for the routine removal of the phenolic compounds from aqueous environmental samples.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The increase in urbanization, population, and industrial growth has led to increased contamination of the environment by discharging various pollutants into the water bodies (Yadav et al., 2020). The chemical and petroleum industries generate a wide variety of highly toxic organic pollutants, which have led to cumulative hazardous effects on the environment (Al-Khalid and El-Naas, 2012). These industries' effluents often contain aromatic organic compounds that are somewhat resistant to natural degradation and persist in the ecosystem, leading to the biggest concern for human society's sustainable development. Organic pollutants represent a potential group of chemicals that can be exceptionally hazardous to human health. Therefore, to maintain ecological balance, improve the quality of the environment, and ensure adequate living and working conditions for present and future generations, new technologies are crucial. (Davidescu et al., 2019).

Phenolic compounds are a class of chemical compounds that consist of a hydroxyl group (-OH) that is directly bonded to a hydrocarbon group. These compounds are pollutants of great environmental concern (Darvishmotevalli et al., 2019).

Phenolic compounds are classified under the main class of the plant secondary metabolites and divided into polyphenols and phenolic acids depending upon the phenolic ring's strength. They are also further divided into multiple sub-groups such as lignins, coumarins, flavonoids, and simple phenols (Abbas et al., 2017). Due to

aromatic structure, phenolic compounds are resistant to natural biodegradation and reported to have a high stability because of benzene ring cleavage difficulty (Hao Li et al., 2019). Phenolics are widely distributed in plant tissues. They exist in aglycones (flavonoids), or glycosides, esters, acyl glycosides in their free form. Many plants are rich in these compounds with excellent health benefits and antioxidant activities (Metsämuuronen and Sirén, 2019). Among the phenolic compounds, phenolic acids are the most common and attractive. They are found in grains, vegetables, fruits and other plants (Xu et al., 2019).

Over the past centuries, phenol has been considered an essential compound in creating an aseptic environment, especially for disinfecting patients, surgical instruments, and operating rooms. Phenol is currently considered a priority pollutant since there is a considerable body of evidence that phenolic compounds have toxic effects on human and animal health even at low concentrations. It has been listed as a hazardous pollutant because of its potential harm to human health (Ibáñez et al., 2012). The toxicity level usually ranges from 9–25 mg/L for both humans and marine creatures (Kulkarni, 2013). These compounds create an unpleasant taste and odor in water sources (Balgooyen et al., 2019). Various regulatory organizations have placed phenols and their derivatives on the top list of the most hazardous chemicals that are difficult to remove and have set a stringent disposal limit for phenols for a sustainable ecosystem (Gami, 2014; Sun, Wang et al., 2015b). For instance, the US Environmental Protection Agency (USEPA) has set a water purity standard of less than 1 ppb for phenol in surface water (Kazemi et al., 2013).

Due to widespread industrial technologies, phenols and their derivatives are generally present in industrial effluents and can be released directly or indirectly into the environment (Akintade et al., 2012; Ayeni, 2012). Given the high toxicity,

extensive use over time, and low biodegradability of phenolic compounds, its removal from wastewater before discharge into the atmosphere is crucial (Gopal et al., 2019).

Various adsorbents have been used to remove phenols from water and wastewater (Magdy and Altaher, 2018; Muhamad et al., 2018; Orimolade et al., 2018; Palembang-prabumulih and Ilir, 2015; Nadavala et al., 2014; Njoku et al., 2013). Existing methods for the removal of phenols are distillation, coagulation, electrocoagulation biological treatment, catalytic oxidation (Sun et al., 2020), ozonation (Wei et al., 2020), solvent extraction, and adsorption methods (Cao et al., 2020; Biglari et al., 2017; Yang et al. 2016; Bazrafshan, 2012). Among these methods, the adsorption technique has been mostly established method because of lower operational costs, easier operation control and most importantly, it is a direct physical method in which molecules of liquids are collected on the surfaces of adsorbent materials (Guo et al., 2020; Abdoli and Bargozin, 2015). Nonetheless, existing methods for removing phenols are generally non-selective, which has led many researchers to look for more selective, cost-effective and efficient adsorbents to eliminate organic pollutants from water and wastewater (Yuan et al., 2020; Qassim, 2013).

Nano-sized materials have drawn considerable interest nowadays due to their unique size and physicochemical properties. They have become one of the most versatile research and development frontiers of modern science. Among them is magnetite (Fe_3O_4) fortified with exceptional sorption ability, separation properties, small size, low toxicity, superior superparamagnetic property and ease of preparation, becoming very popular and promising adsorbent materials (Orimolade et al., 2018; Wang et al., 2016). While the high area-to-volume ratio of nanomaterials contributes to increased capacity with associated improved performance, the occurrence of

aggregation, non-specificity and low stability can restrict the use of these nanotechnologies due to lack of functionality (Guerra et al., 2018).

Over the past decades, synthetic polymeric adsorbents have emerged as an efficient adsorbent that effectively traps many ubiquitous organic pollutants in an aqueous solution. Due to its good mechanical strength, adjustable surface chemistry, pore size distribution, feasibility for regeneration and repeated use under mild conditions, as well as the potential recovery of some valuable organic compounds from wastewater (Xiao et al., 2012). Moreover, it is a cost-effective alternative for removing organic pollutants (Issabayeva et al., 2018). Many polymeric adsorbents have been exploited through chemical modification by crosslinking/grafting some special functional groups in order to obtain better adsorption capacity and selectivity for some persistent organic pollutants (Awad et al., 2019; Surikumaran et al., 2015; Surikumaran et al., 2014a; Folch-Cano et al., 2014; Raoov et al., 2013; Xiao et al., 2012). Cyclodextrin (CD), characterized as supramolecular host compounds, belongs to a series of cyclic oligosaccharides consisting of 6-8 glucose units, commonly used as water-soluble functional units.

Interestingly, the CD is used to synthesize polymeric materials by numerous approaches (Arslan et al., 2019). CD and its derivatives contained a hydrophobic inner cavity and hydrophilic external cavity capable of forming inclusion complex through host-guest interactions. Therefore, it can be utilized as a molecular recognizer (Gong et al., 2016). Cyclodextrins have attracted attention in water treatment in the past years owing to several relevant advantages (Pellicer et al., 2019). For these reasons, in the present study, CD-based polymeric adsorbent materials for removing selected phenolic compounds (bisphenol A, 2,4-dinitrophenol and 2,4- dichlorophenol) will be exploited.

1.2 Problem statement

Phenol has been listed as a priority pollutant by various regulatory agencies, such as the United States Environmental Protection Agency (USEPA) and Canada's National Pollutant Release Inventory (NPRI) (Villegas et al., 2016). Bisphenol A (BPA) is one of the most voluminous synthesized organic compounds in the world. This compound is the building block of polycarbonate plastics and is commonly used for food and beverage packaging (Rubin, 2011). Bisphenol and its derivatives are commonly found in products used in everyday use, such as packaged goods, baby bottles, toys. Exposure to this harmful chemical can pose a grave threat to human health. Studies have also shown that these products are highly susceptible to bisphenol leaching when exposed to detergents, acidic fluids and heated at high temperatures (Samineni, 2017).

Given its widespread use and annual global production of approximately 6.8 million tons, it is unsurprising that BPA has evolved to become a significant environmental contaminant that leaches into water and soil (Al-Hiyasat, 2017). BPA is an endocrine-disrupting compound that may replicate the hormones in the body and interact with the human body's natural hormone activity and influence human safety. (Kabir et al., 2015). BPA has been reported to affect human growth during the embryonic stage and may be carcinogenic (Ayeni, 2012). Legislative bodies like the U.S. Environmental Protection (U.S.E.P.A) and the European Food Safety Authority (E.F.S.A) have set a restricted consumption limit of 25-50 μg BPA/kg body weight per day.

2,4-dinitrophenol (2,4-DNP) belongs to a class of alkyl dinitrophenols known to be the group of persistent organic compounds in the environment. Nitrophenols are extensively detected in industrial effluent streams due to the diverse applications of 2,4-DNP as a raw material for specialty chemicals or intermediate in textiles, dyes, pesticide and pharmaceutical industries (Bagal et al., 2013). This chemical is found in industrial effluent sources due to its vast applications. Its carcinogenic nature poses a considerable health risk, and 2,4-DNP will restrict cell growth even at significantly lower concentrations (1 ppm) (Bagal et al., 2013; Cao and Shiraishi, 2010).

On the other hand, Chlorophenols are a subgroup of very toxic phenolic compounds with high chemical oxygen demand and low biodegradability (Mohammadi et al., 2017). They have detrimental effects on living organisms and endanger human health even at low concentrations. 2,4-dichlorophenol (2,4-DCP) is a persistent contaminant frequently found in agricultural sites, water disinfected by chlorination, and pulp and paper mill (Li et al., 2015). Since chlorophenols cause kidney, liver, pancreas damage, weakening the central nervous system, and denature protein molecules, USEPA has placed them in the category of high-risk pollutants (Zin et al. 2018) and classified phenol and its derivatives as the 11th of the 126 chemicals in the class of high-risk pollutants (Gholizadeh et al., 2013). The environmental protection agency (EPA) recommends that 2,4-DCP concentration in drinking water should not exceed 0.03 mg/L (Gholizadeh et al., 2013) and the World Health Organization (WHO) has also recommended 1 μg/L and 1 mg/L as its maximum permissible concentrations in drinking water and wastewater discharged into surface water, respectively (Basri et al., 2009).

Currently, the removal of these pollutants remains a challenge to researchers. Several methods such as membrane separation, photocatalytic degradation, and adsorption have been developed to remove these pollutants. However, these methods are expensive, non-selective and require pre-treatment. Therefore, magnetic molecularly imprinted polymer (MIP) has been chosen in this study due to its advantages such as excellent selectivity, ease of preparation and reusability.

1.3 Objectives

This research aims to synthesize cyclodextrin-based polymeric adsorbents for the removal of phenolic compounds from aqueous solutions.

The specific objectives of this study are:

1. To develop a new adsorbent of magnetite methacrylic acid functionalized β-cyclodextrin (Fe_3O_4@MAA-βCD) for the removal of selected phenolic compounds (2,4-DCP, BPA and 2,4-DNP).
2. To study the adsorption behavior between Fe_3O_4@MAA-βCD and selected phenolic compounds in water samples.
3. To develop a new adsorbent using methacrylic acid (MAA) and methacrylic acid functionalized β-cyclodextrin (MAA-βCD) as co-monomers in magnetic molecularly imprinted polymer synthesis (MMIP MAA-βCD) for the removal of BPA in water samples.
4. To study the interaction mechanism between MAA-βCD monomer and BPA through inclusion complex formation.

1.4 Scope of the study

This work is divided into five chapters. **Chapter 1** is the introduction which describes the general background of the study. In **Chapter 2,** relevant literature related to the study was reviewed in detail. **Chapter 3** is the methodology chapter chemicals and reagents that have been employed in these studies were highlighted. The synthesis of the adsorbent materials and preparation of the inclusion complex was also described. **Chapter 4** is designated as the results and discussion section. Herein, the chapter is sub-divided into three parts. This chapter encompasses the discussion of results obtained in this study. **Part I**, the characterization of the synthesized adsorbents, is discussed. **Part II** encompasses the application of Fe_3O_4@MAA-βCD to remove BPA, 2,4-DNP and 2,4 DCP in an aqueous solution. Several variables influencing the adsorption efficiency of the adsorbent were optimized. The method was validation, removal study using real sample and reusability studies were also discussed. In part **III**, the application of MMIP MAA-βCD for the removal of BPA in an aqueous solution was discussed in detail. First, preliminary sorption studies were discussed. Likewise, selectivity study, method validation, real sample analysis, and reusability study were also discussed.

Additionally, this study was compared to other relevant studies in the literature in this chapter. Interaction between MAA-βCD monomer and BPA through inclusion complex formation is discussed. Finally, in **Chapter 5,** the conclusion and recommendations for future studies were discussed.

CHAPTER 2

LITERATURE REVIEW

2.1 Phenolic Compounds

Phenols are categorized as one of the most serious environmental contaminants discharged from industrial activities such as oil refineries, plastic plants, dyes, pesticides, pharmaceuticals, coal conversion, coking, and petrochemical (Gami, 2014).

Phenolic compounds are usually discharged from these industries and accumulated in the environment as a high-priority pollutant (Abdelkreem, 2013; Torabi et al., 2016). Untreated discharge of these compounds can lead to serious health risks to humans, animals and aquatic systems (Sun et al., 2015). International regulatory bodies have set strict discharge limits for phenols for a sustainable environment (Sun et al., 2015; Villegas et al., 2016). Phenol has side effects on health that can be both chronic and acute. Long-term exposure will lead to irregular respiration, muscle weakness, tremor, coma and respiratory arrest at lethal doses in humans. Human exposure to phenol irritates the skin, eyes and mucous membranes (Gupta et al., 2015). Chronic symptoms due to phenol exposure may include anorexia, weight loss, diarrhea, vertigo, salivation, and dark urine coloration (Gupta et al., 2015). Chronic exposure to phenol causes inflammation in animals of the gastrointestinal, central nervous systems, liver, kidney and cardiovascular tissue (Mohammadi et al., 2015; Villegas et al., 2016). Figure 2.1 shows the structures of phenolic compounds considered priority pollutants by USEPA.

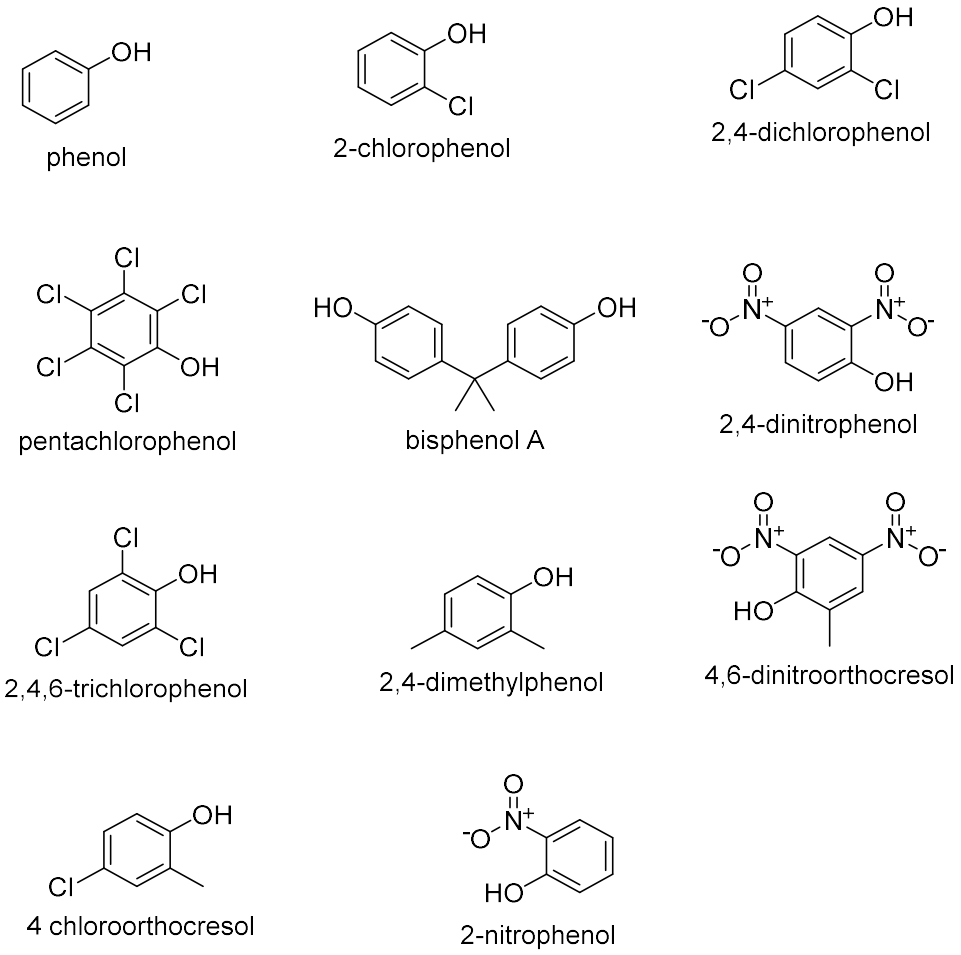

Figure 2.1: Structures of phenolic compounds considered priority pollutants by USEPA

2.2 The occurrence of phenolic compounds in Malaysia

Pollution is a global environmental problem and is becoming a major concern, especially in Malaysia (Abd Gami et al., 2014; Arif et al., 2013). Common xenobiotic contaminated environments of phenols can result in the bioaccumulation of phenols in the ecosystem through anthropogenic natural or process activities such as effluent/wastewater discharge, mining, industrial and agricultural activities.

The rapid urbanization and growth of the population have led to both the ever-increasing demand for water consumption and, consequently, increased water

pollution levels in Malaysia (Torabi et al., 2016). The tremendous increase in industrialization in Malaysia over the past 20 years has been the most significant contributor to this problem, in addition to the natural process of plant decay by synthesizing chlorinated phenols such as tannin as a secondary metabolite naturally. The phenolic compound exists in the environment through several sources, for example, phenoxy herbicides like 2, 4-dichlorophenoxyacetic acid (2, 4-D) and phenolic biocides like pentachlorophenol (PCP) that are found within the environment due to numerous human activities (Mohamed et al., 2011). In Malaysia, the Department of Environment has reported that 1,709 metric tons of phenol and phenolic wastes were generated in 2005 (Arif et al., 2013). The National Guidelines for Raw Drinking Water Quality reported that the maximum permissible limit for phenolic compounds is 0.002 mg/L. Many groundwater wells in Malaysia have phenolic levels that surpass this limit (Arif et al., 2013). Thus, indicating widespread pollution caused by phenol and its derivatives.

2.3 Cyclodextrin (CD) based polymeric adsorbents

Cyclodextrins (CDs), characterized as a supramolecular host, belongs to a series of cyclic oligosaccharides consisting of several α-1, 4-linked-glucopyranose units. Due to its structure, physicochemical properties, chemical stability, high reactivity and excellent selectivity toward organic compounds and metals, CDs have gained a great deal of interest as low-cost adsorbents of choice for wastewater treatment (Huang et al., 2013; Karoyo and Wilson, 2015). It is widely known that CDs can form inclusion complexes through host-guest interactions with a wide range of organic compounds in its hydrophobic cavity (Rajbanshi et al., 2018). This ability to encapsulate molecules is commonly used in many industrial products, machinery and

analytical methods. CD-based polymeric adsorbents have attracted much attention due to their high affinity for various organic pollutants, low cost, and simple design (Xu et al., 2019). CDs and their derivatives were also used as building blocks to create a wide range of polymeric networks and assemblies, which have been used for a wide range of applications (Curtin-Gomez et al., 2020). Many polymeric materials such as hydrogels, nano/microparticles and micelles are studied for different applications (Vermonden et al., 2009). Many methods on the synthesis and characterization of CD polymers have been published in the literature (Maruthapandi and Gedanken, 2019). Such synthetic methods are widely divided into two categories: (i) the use of cross-linkers to interconnect the hydroxyl groups of CDs into a polymer network capable of producing soluble or insoluble products, gels, extended and branched polymer networks; and (ii) grafting (covalent bonding) CDs to the pre-existing polymer main chain (Danquah, 2017). Current studies on the design of porous βCD polymers have shown promising sorption results where diisocyanate cross-linkers are used (Anne et al., 2018; Li et al., 2016; Mohamed et al., 2011; Raoov et al., 2013).

2.4 Molecularly imprinted polymers

Molecularly imprinted polymers (MIPs) are artificial polymers with artificially generated recognition sites that can specifically rebind the target molecules selectively in the presence of other closely related compounds (Madikizela and Tavengwa, 2018; Joke Chow and Bhawani, 2016). MIPs are obtained by polymerizing functional and crosslinking monomers around the target molecule (template) in a complementary fashion resulting in a highly crosslinked three-dimensional network (Belbruno, 2019; Moein et al., 2019; Pratiwi et al., 2018). When the template is removed, it creates recognition binding sites in the polymer matrix. Hence the template/target analyte of

interests can be recognized from complex environmental samples (Sikiti et al., 2014). Over the years, MIP has gained widespread attention and has become attractive in many fields, such as purification and separation, chemosensing/ biosensing, artificial antibodies, drug delivery, catalysis, and degradation. Due to their high physical and thermal stability, structure predictability, recognition specificity, simple preparation, remarkable robustness, and low cost (Sajini et al., 2019). However, conventional MIP preparation techniques exhibit some drawbacks such as limited site accessibility to the target molecules, low rebinding capacity, slow mass-transfer rate and incomplete template removal (Li et al., 2018). To overcome these drawbacks, surface molecular imprinting technology has been developed in recent years. Recently, MIP-functionalized magnetic composites have become a hotspot due to its bifunctional property of the selectivity for the target molecules and the rapid magnetic response (He et al., 2014).

2.5 Consideration in the synthesis of selective MIP

In MIP synthesis, several factors such as monomer selection, template selection (Trehan et al., 2013; Andersson et al., 1999), crosslinker (Cai and Gupta, 2004), and solvent selection (Masque et al., 2001) need to be considered since they can affect MIP morphology, properties, and efficiency.

2.5.1 Functional monomers

Monomers are materials that shape binding sites on the imprinted polymer, resulting in its interaction with the template molecule (Asman, 2015). The critical step in the synthesis of MIP is the prearrangement of monomers in the presence of a template molecule. The monomers are selected to interact with the template by either

non-covalent interactions or reversible covalent interactions or metal ion-mediated interactions. The monomers' structure and concentration as part of imprinting protocol are typically selected from knowledge or published records. The monomer is used to build sample high-precision cavities. To maximize the MIP preparation, the template molecule's functionality must be balanced in a complementary manner to the functionality of the monomer, for instance, H-bond donor with H-bond acceptor. The ratio of 1:4 and upward: for monomer is suitable for non-covalent imprinting to some degree (Cormack and Elorza, 2004). Figure 2.2 demonstrates typical functional monomers such as carboxylic acids (acrylic acid, methacrylic acid, vinyl benzoic acid), sulphonic acids (acrylamide2-methylpropane sulphonic acid), and heteroaromatic bases (vinyl pyridine, vinyl imidazole). Methacrylic acid is widely used as a monomer because of its capacity to function both as hydrogen bonds or protons a donor and acceptor. (Hart et al., 1999). There were efforts to find new monomers that would suit the MIP planning. βCDs have acquired a broad interest in molecular imprinting as functional monomer owing to its unique structural properties. However, cyclodextrins or their derivatives can be used as monomers in various types of polymerization techniques such as reversible addition-fragmentation chain transfer (RAFT), ring-opening polymerization (ROP), free-radical polymerization, anionic polymerization, cationic polymerization, nitroxide mediated radical polymerization (NMP) and metathesis polymerization (Seidi et al., 2019). Typical βCD monomers can form interactions such as hydrogen bonding, van der Waals, hydrophobic or electrostatic interactions and inclusion complex formation.

Figure 2.2 highlights the structures of some typical functional monomers used in the MIP synthesis.

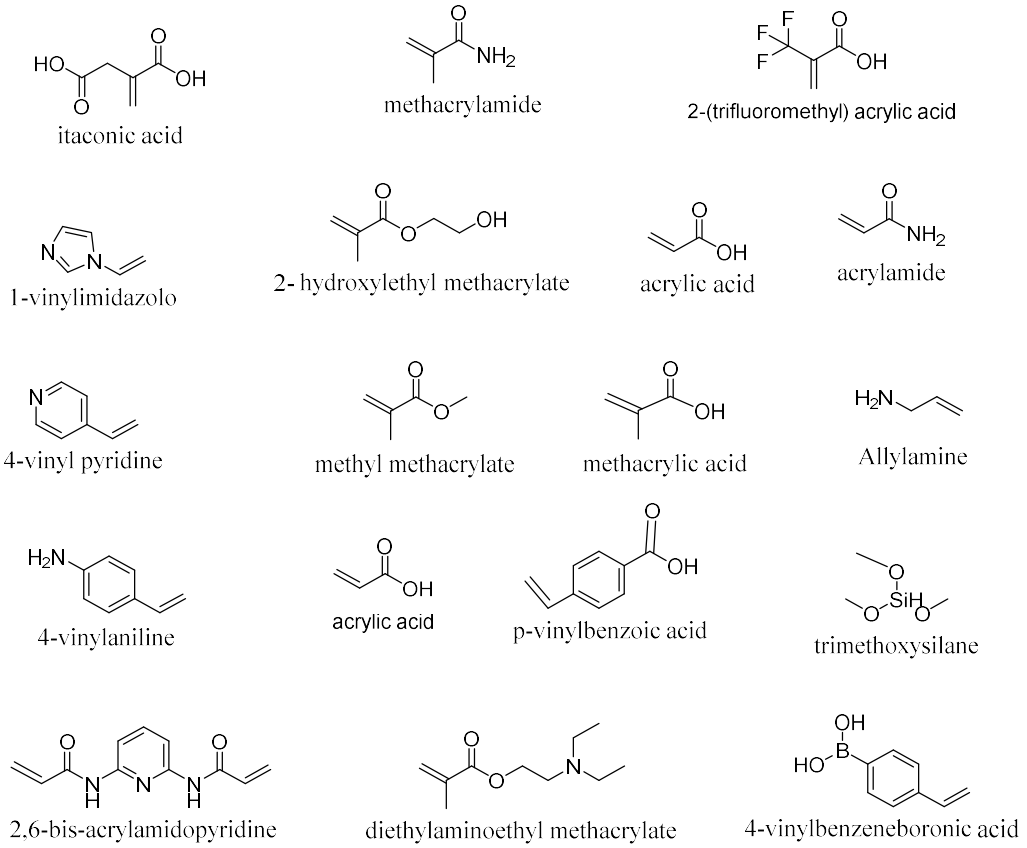

Figure 2.2: Structures of some typical functional monomers used in MIP synthesis.

2.5.2 Crosslinker

As part of the imprinting process, the crosslinker is used to achieve high selectivity for the MIP. The cross-linker is vital for controlling the polymer matrix's morphology. It stabilizes the imprinted binding sites and provides mechanical stability to the polymer matrix to maintain its molecular recognition capacity (Asman et al., 2015b; Sellergren, 1999).

Multiple crosslinkers were tested to synthesize molecularly imprinted polymers but by far, EGDMA and TRIM are the most extensively used crosslinkers. Exposure to permanently porous (microporous) materials with outstanding mechanical toughness is commonly utilized with large crosslink ratios. Figure 2.3 depicts the structures of common crosslinkers used in MIP synthesis. Among these cross-linkers, divinylbenzene (DVB) was the first crosslinker employed with the purpose of modern MIP preparation (Wulff and Sarhan, 1972). A later study by Wulff et al. (1987) compared the DVB with three other candidates: EGDMA, trimethylolpropane trimethacrylate (TRIM) methylene bis-acrylamide. Their results suggested that the EGDMA was the best cross-linker and polymer fabricated using 70-95 % of EGDMA produced the MIP with the best performance for racemic resolution (Wulff et al., 1987). Nowadays, EGDMA has become the most widely used cross-linker. New crosslinking systems have been proposed in several studies. TRIM offers more rigidity, structure order and multiple binding sites to polymers than EGDMA (Wei et al., 2015). Some researchers consider that crosslinker has a detrimental impact on polymers' physical properties and less on the interactions between the template and the monomer (Ye et al., 2000). The cross-linker form was observed in another analysis to have a particular impact on the yield and final size of MIP nanoparticles (Yoshimatsu et al., 2007). Low yield polydisperse MIP particles were obtained when divinylbenzene was used as a crosslinker, while TRIM (90%) and uniform nanoparticles achieved high yield. Recently, many researchers have synthesized MIP using various commercially available cross linkers such as DVB (Tan et al., 2020) N, N-methylene bis acrylamide as a crosslinker for the synthesis of surface molecularly imprinted thermo-sensitive polymers based on light-weight hollow magnetic microspheres for specific recognition of bovine serum albumin (Zhou et al., 2019). In

another study, N-O-bismethacryloyl ethanolamine was used as a crosslinking monomer to prepare MIP to detect highly toxic polycyclic aromatic hydrocarbons (PAHs) in seawater (Krupadam et al., 2014). EGDMA has also been reported s cross linker in numerous studies(Díaz-Álvarez et al., 2016; Orimi et al., 2020; Viveiros et al., 2018).

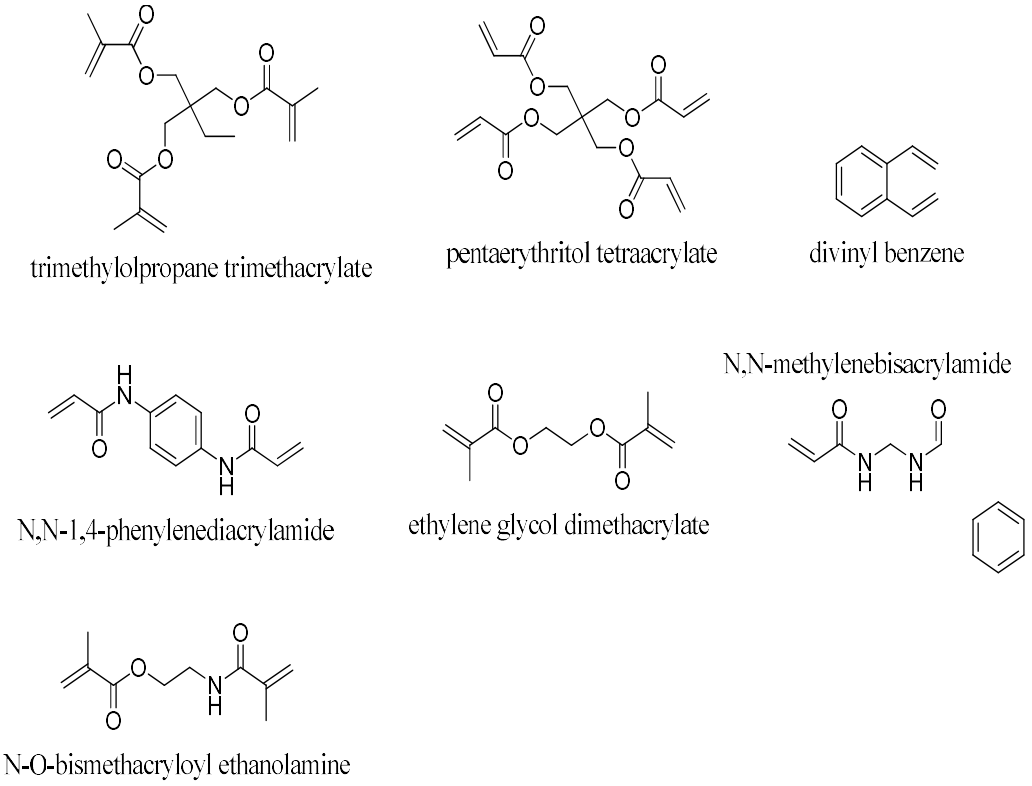

Figure 2.3: Structures of common crosslinkers used in MIP synthesis.

2.5.3 Template

The species that serve as the template should have the most profound impact on the imprinting process results. The success of the molecular imprinting process is determined by the interaction between the functional monomer and the template. Ideally, those interactions should be strong so that the recognition mechanism after the polymer synthesis could be enhanced. Suitable species should be chosen carefully to

produce the highest number of well-defined binding sites (Engenharia et al., 2016). For all molecular imprinting structures, architecture is important because it guides the assembly of different groups attached to different monomers (Ganjali et al., 2015).

Unfortunately, not all templates are suited explicitly for templating and for several purposes. Regarding conformity with free-radical polymerization, under polymerization circumstances, the templates should be chemically inert. An alternate form of imprinting will also be explored if, for whatever cause, the template may be involved in radical reactions or is deficient under polymerization conditions (Saliza et al., 2015).

2.5.4 Porogen (solvent)

The porogenic solvent serves to bring all the components (template, monomer, cross-linker, and initiator) in the polymerization into one phase. It plays a significant role in the formation of the porous structure of MIP. The nature and level of the solvent determine non-covalent interactions' strength, impact polymer morphology, and directly influence the MIP performance. The porogenic solvent should produce large pores to guarantee good flow through the resultant MIP properties (Ganjali et al., 2015). An increase in the volume of the solvents will broaden the pore volume of the polymer. Therefore, the solvent is referred to as the "porogen." Moderately low polarity solvents are utilized in MIP synthesis to lessen the interferences during complex formation between the imprint molecule and the monomer (Vasapollo et al., 2011; Yan and Row, 2006). In many reports, efficient MIPs have been synthesized in polar solvents because robust template- monomer interactions have been observed. For instance, water-compatible imprinted polymer with a strong affinity for a polar 1-methyladenosine template has been synthesized in acetonitrile/water and successfully

used as solid-phase extraction (SPE) sorbent to extract analyte from spiked human urine samples (Scorrano et al., 2010).

2.5.5 Initiator

The initiator is used to trigger the initiation of the polymerization process. Numerous chemical initiators with various properties can be utilized as the radical source in free radical polymerization. Typically, initiators are used at low levels compared to the monomer, for instance, 1 wt. % or 1 Mol % concerning the total number of polymerizable double bond moles (Cormack and Elorza, 2004). The rate and method of decomposition of an initiator to radicals can be triggered and controlled from numerous points of view, including heat, light, and chemical/electrochemical means, depending upon its chemical nature. The commonly used initiators are benzoyl peroxide, azobisisobutyronitrile, azobisdimethylvaleronitrile, and 4,4'- azo (4-cyanovaleric acid) and their structures are shown in Figure 2.4

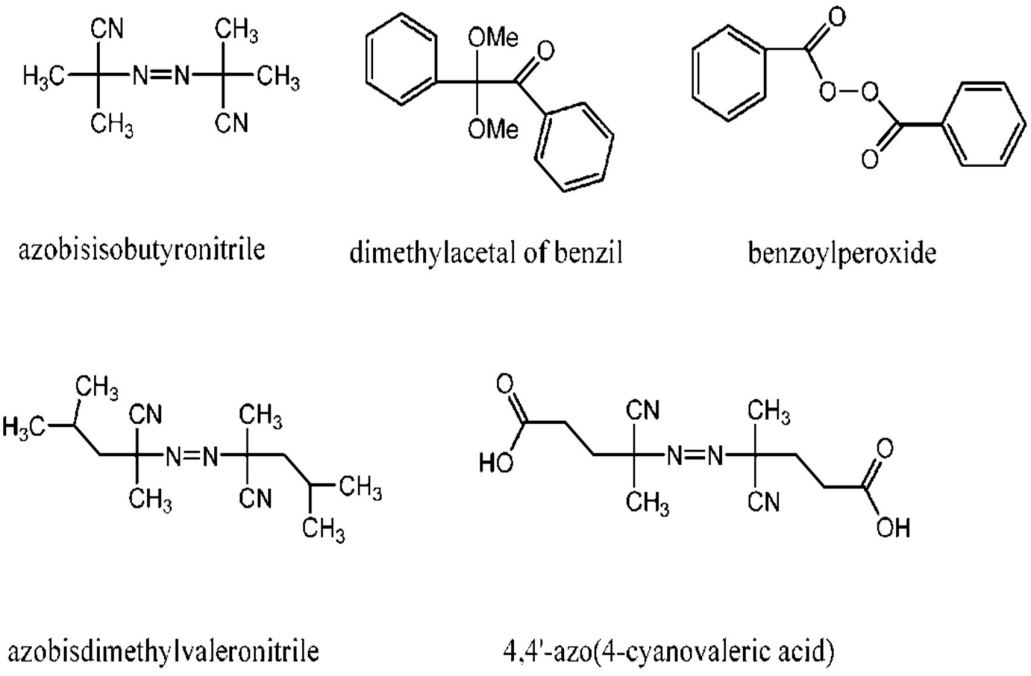

Figure 2.4: Structures of typical initiators used in MIP synthesis.

2.6 Methods of molecularly imprinted polymers Synthesis

Specific approaches to polymerization have been used in the processing of MIPs over the past decades. The most commonly used methods include bulk, precipitation, suspension, multi-step emulsion, and sol-gel polymerization techniques (Pérez-Moral and Mayes, 2004). Each MIP synthesis method has its inherent disadvantages that need to be considered based on the potential application of the MIP before selecting the preferred method to be used. In a nutshell, MIP polymers' selectivity and specificity require modification by careful selection of experimental conditions.

2.6.1 Bulk polymerization

MIP can be synthesized to match the final application of choice in several physical forms. This approach is the most popular since Wulff and his associates first prepared the MIP (Wulff, 1973). All the materials are dissolved in a limited amount of an appropriate solvent which often acts as a porogen rather than a photochemically regulated or thermally managed polymerization. In this method, a limited porogen volume (about 10 mL solvent) is used to create a single stable monolith. The insoluble polymer monolith is broken and interfering with minute micrometric pieces. A sample is collected using a simple solvent extraction technique. Since the porosity of the MIP obtained by bulk polymerization can be modified using specific solvents or concentrations of monomers, solvents' choice is limited to template solubility and stability of the formed template – monomer complex. The bulk polymerization method is rapid and straightforward preparation needs no sophisticated or expensive instrumentation.

Nevertheless, after polymerizing the bulk polymer, it has to be crushed, ground and sieved to an appropriate size. After grinding, the polymer with irregular shape and size is obtained. The size range is typically between 5-10 μm and can be tuned with respect to the final application (Bates, 2016). The main downside to this approach comes from the destruction of some high-affinity binding sites, which reduces the binding capacity of the MIP (Fitzhenry, 2011). Many MIP fiber coatings and various SPME adsorbents have been identified with bulk polymerization (Piri-Moghadam et al., 2017; Xu et al., 2013). Because particle heterogeneity can be a problem for some MIP applications, various polymerization methods have been implemented to achieve standardized beads and microsphere scale (Filipa and Lobo, 2015).

2.6.2 Precipitation polymerization

Precipitation polymerization is the second most widely used strategy after bulk polymerization. It starts in the continuous process as a homogeneous device, where the monomer and the initiator are wholly soluble. However, the produced polymer is insoluble in the chosen solvent and thus precipitates microspheres (Cai et al., 2013). This method can obtain MIP with micro-spherical shapes and a more uniform size, which offers a higher active surface area by manipulating its compositions. This technique is like bulk polymerization, but it requires a much more complex mixture of monomers due to working with an excess of solvent. Thus, the polymer grows in individual spherical nanoparticles, with high yield and uniform binding site distribution. The downside of this method is that it requires a more generous amount of solvent than that used in the conventional method (approximately <5% (Jinfang Wang et al., 2003) of the total volume of monomer loading relative to the solvent), producing an average particle diameter around 0.2–0.3 μm (Orowitz et al., 2020).

2.6.3 Suspension polymerization

Suspension polymerization is one of the relatively easy processes used to prepare imprinted supports. In conventional suspension polymerization, water is used as a continuous phase to suspend droplets of pre-polymerization mixtures (template molecule, functional monomer, cross-linker and initiator) in the presence of a stabilizer or surfactant (Chaudhary and Sharma, 2019). The least sophisticated heterogeneous polymerization starts with the formation of organic and aqueous phases separately. The organic phase to aqueous phase volume ratio usually varies from 0.1 to 0.5 or even more. The organic phase is prepared by the addition of monomer, initiator, crosslinker molecules and diluents.

On the other hand, surfactant and stabilizer are dissolved in distilled water to make an aqueous phase. Monomer droplets are dispersed in an aqueous phase with the addition of surfactant molecules (sodium dodecyl sulfate/sodium sulfate) and stabilizer (methylcellulose/PVA/ gelatin) to prevent amalgamation and breakage during polymerization. The monomer phase is suspended in the medium in small droplets employing a stirrer and a suitable suspension agent. Also, a monomer soluble initiator (free radical) is added for both initiation and chain-growth mechanism within the monomer droplets; however, monomer and initiator are insoluble in the medium (Minami et al., 2017). The initiation and propagation mechanism occurs inside the monomer droplets. The product is collected by conventional filtration and washed to eliminate stabilizer and other contaminants

Suspension polymerization does not involve mechanical grinding, which results in aggregates of spherical particles; if the medium is sufficiently diluted, microspheres of uniform sizes are obtained. This polymerization strategy is one of the most straightforward and most used approaches for MIP bead growth. (Filipa and Lobo, 2015; Mosbach and Mayes, 1996). MIP beads have been commonly prepared via suspension polymerization, and templates suitable for this system include metal ions, drugs and proteins. In the meantime, it was found that this approach was limited to particular compositions, that the MIP beads prepared may have a broad size distribution (Gomes et al., 2017). The downside of this method is that stabilizer or surfactant, required for the formation and stabilization of droplets, may interfere with the interactions between the template molecule and functional monomer. While the results showed some promise, the preparation needs careful handling, and beads eventually reduce the imprinted polymer volume per unit column (Mayes and Mosbach, 1996).

2.6.4 Emulsion polymerization

This technique is more complicated than the bulk polymerization strategy; it is free radical polymerization. The liquid monomer is dispersed into an insoluble material resulting in an emulsion formation (Roy, 2006). An oil-in-water emulsion is the most popular type of emulsion polymerization in which monomer droplets (the oil) are emulsified (with surfactants) in a continuous water phase (Kumbar and Deng, 2014). The particle size of the MIPs produced is said to range from 50–500 nm. Thanks to its large specific surface area and high adsorption capacity, it has been widely used in MIP preparation.

2.6.5 Multistep Swelling polymerization

MIP developed a multi-step polymerization technique for swelling by Hosoya et al. (1996). This requires multiple swelling stages over the initial particles. The particles in their interior phase absorb the emulsion. They are then transferred to another emulsion with the imprinting mixture, including the monomers and the template in the oil phase, before polymerization continues. In this case, the polymerization medium's continuous phase is water. This process generates monodisperse particles in a size range of (2–50 μm) with significant control over the final particle size number. Various commercially important polymers were produced using this approach (Filipa and Lobo, 2015). A uniformly formed MIP prepared using the multi-step swelling strategy with methacrylic acid as a monomer and d-chlorpheniramine as a template showed superior selectivity of the template compared to its corresponding NIP and slight recognition to its structurally related compounds (Haginaka and Kagawa, 2002). In another work, MIP based on MAA was prepared non-covalently using a two-step swelling method with diaminonaphthalene isomers or

chiral amide derived from (S)-α-methyl benzylamine, as the interest analyte was shown to improve chiral recognition (Hosoya et al., 1996). Though the polymer particles are relatively monodispersed in size and shape and suitable for chromatographic applications, it requires complicated procedures and reaction conditions. The requirement for aqueous suspensions (emulsions) used for this strategy may also affect the imprinting resulting in a decline in selectivity.

2.6.6 Sol-gel polymerization

The sol-gel polymerization method allows water-compatible MIP synthesis, reducing the possibility of blockage and distortion of imprinted cavities due to changes in the degree of swelling. This process is used to prepare advanced inorganic and organic-inorganic hybrid material with even porosity, good selectivity, high thermal and chemical stability. This approach has been developed for different analytical separation. In this method, a liquid colloidal solution, known as the sol, is transformed into a solid" gel" matrix. The technique involves two essential steps: the hydrolysis of the sol-gel precursor and the hydrolyzed products' polycondensation and other active sol-gel moieties in the medium. In the polycondensation process, sol-gel active organic ligands are randomly integrated into the growing inorganic network (Kabir et al., 2013). Numerous factors are crucial for influencing the performance of a sol-gel method, such as type and nature of the precursors, precursor: water ratio, type and concentration of catalysts (acid, base, fluoride), pH of the sol solution, organic solvent: precursor ratio, temperature, humidity, and post-gelation aging conditions (Kabir et al., 2013).