MOLECULARLY IMPRINTED SILICA MATRIX IN DETECTION OF CREATININE

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by

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To my eternal cheerleader, Aunt Lily: I enjoyed our delightful and intriguing chats. My persistently supporting and passionate aunt who always eager to know what I was doing and how I was proceeding, although it is likely that she has never grasped what it was all about. Thank you for helping me making it through.

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Florence Chan

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

Symbol	Description	Unit
A_0	Initial analogue concentration in solution	mg/L
A_e	Equilibrium analogue concentration in solution	mg/L
C_0	Initial creatinine concentration in solution	mg/L
C_a	Equilibrium creatinine concentration on the absorbent	mg/L
C_e	Equilibrium creatinine concentration in solution	mg/L
K_D	Distribution coefficient	-
$\Delta G°$	Gibb's free energy	J/mol
$\Delta H°$	Enthalpy	J/mol
$\Delta S°$	Entropy	J/K
q	Adsorption capacity	mg/g
R	Ideal gas constant	J/mol.K
T	Absolute temperature	K
V	Volume of adsorption solution	mL
W	Weight of imprinted polymer	g

LIST OF ABBREVIATIONS

2-pyr	2-pyrrolidinone
4-MDBT	4-methyldibenzothiophene
4-vpy	4-vinylpyridine
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
BET	Brunauer-Emmet-Teller
BT	Benzothiophene
BUN	Blood Urea Nitrogen
Cr	Creatine
Cre	Creatinine
DBT	Dibenzohiophene
DVB	Divinylbenzene
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
MIP	Molecularly Imprinted Polymer
MIT	Molecular Imprinting Technology
NIP	Non-imprinted Polymer
PCr	Phosphocreatine
SEM	Scanning Electron Microscope
TEOS	Tetraethoxysilane
UV	Ultraviolet
UV-Vis	UV-Visible Spectrophotometer
β-CD	β - cyclodextrin

ABSTRAK

Kepekatan serum kreatinin secara umumnya ditafsirkan sebagai ukuran kadar penapisan glomerular (GFR) dan digunakan sebagai indeks fungsi buah pinggang dalam amalan klinikal. Kaedah tindak balas Jaffe yang digunakan untuk mengukur kepekatan kreatinin serum tertakluk kepada pelbagai gangguan dan oleh itu kehilangan spesifikasinya. Dalam usaha untuk menambahbaikkan kaedah analisis, polimer molekul bercetak (MIP) dikaji kerana keupayaannya untuk mengenal pasti dan menjerap hanya molekul yang terpilih. Berdasarkan tetraetoksisilan (TEOS) sebagai monomer dan Al^{3+} sebagai pemaut, MIP telah disentesis melalui kaedah sol-gel untuk penjerapan terpilih kreatinin (Cre). Keputusan mikroskop elektron pengimbas (SEM) mendedahkan bahawa MIP mempamerkan struktur yang lebih poros berbanding dengan polimer tidak bercetak (NIP), manakala analisis keluasan permukaan dan keporosan mendedahkan bahawa keluasan permukaan MIP (570.32 m^2g^{-1}) adalah lebih besar daripada NIP (412.47 m^2g^{-1}). Ujian pengikatan semula dilakukan pada suhu 30^oC selama 24 h untuk menilai keupayaan penjerapan kedua-dua MIP dan NIP. MIP didapati mempunyai faktor pencetakan yang baik (1.34 ± 0.26). Kesan suhu dan pelarut proses penjerapan telah dikaji. Parameter termodinamik MIP dan NIP diperolehi daripada penjerapan yang dijalankan pada pelbagai suhu (nilai negatif ΔG^o, nilai-nilai positif ΔH^o dan ΔS^o), menunjukkan bahawa proses penjerapan bagi MIP adalah spontan, endotermik, dan entropi meningkat. Kesan pelarut telah dijalankan dengan air deionized (kekutuban tertinggi), metanol, etanol dan 2-propanol (kekutuban terendah) sebagai pelarut. Interaksi pelarut dan zat terlarut memainkan peranan penting dalam menentukan jumlah Cre terjerap oleh MIP. Pelarut yang mempunyai kekutuban yang lebih tinggi (air) menghasilkan lebih banyak ikatan hidrogen dengan

Cre. Ini menyumbang kepada daya tarikan yang lebih kuat antara Cre dengan pelarut, oleh itu, kemungkinan Cre untuk dijerap pada permukaan MIP menjadi kurang. Di samping itu, ujian selektif telah dilakukan untuk menilai keupayaan diskriminasi MIP. Hasilnya menunjukkan bahawa MIP mempunyai afiniti yang lebih tinggi terhadap templat, Cre berbanding dengan analognya (Cr dan 2-pyr) dalam penjerapan komponen tunggal. Dalam larutan binari, pemilihan MIP untuk Cre berbanding dengan 2-pyr adalah tinggi (3.30 ± 0.34). Namun begitu, pemilihan untuk Cre berbanding dengan Cr adalah rendah (0.46 ± 0.12). Kesimpulannya, MIP berasaskan Cre telah berjaya disintesis, dan boleh digunakan sebagai alat diagnostik untuk fungsi buah pinggang.

ABSTRACT

Serum creatinine concentration is broadly interpreted as a measure of the glomerular filtration rate (GFR) and is used as an index of renal function in clinical practice. The traditional Jaffe's reaction used to measure the serum creatinine concentration, is subjected to interferences and hence lose its specificity. In order to improve the analysis method, molecularly imprinted polymer (MIP) is studied due to its superb capability of recognizing targeted molecules selectively. Based on tetraethoxysilane (TEOS) as monomers and Al^{3+} as cross-linker, a molecularly imprinted silica matrix was synthesized via sol-gel method for the selective adsorption of creatinine. SEM results revealed that MIP exhibited more porous structure compared to the non-imprinted counterpart, while nitrogen adsorption-desorption analysis disclosed that the specific surface area of MIP (570.32 m^2g^{-1}) was larger than that of NIP (412.47 m^2g^{-1}). Rebinding test was performed at 30°C for 24 h to assess the adsorption ability of both MIP and NIP. The MIP was found to have good imprinting factor (1.34 ± 0.26) over the NIP. The effects of temperature and solvent on the adsorption process were studied. The thermodynamic parameters of MIP and NIP were determined from the adsorption conducted at various temperatures (negative value of $\Delta G°$, positive values of $\Delta H°$ and $\Delta S°$), indicated that binding system for MIP was spontaneous, endothermic, and entropy gained. The solvent effect was carried out with deionized water (highest polarity), methanol, ethanol and 2-propanol (lowest polarity) as solvent. The solvent-adsorbate interaction played important role in determining the amount of Cre adsorbed by the MIP. Solvent with higher polarity (water) forms more hydrogen bonds with Cre, leading to stronger interaction forces with it, thus, reduced the possibility for Cre to be adsorbed. Furthermore, selectivity

tests were also been performed in this work to evaluate the discrimination ability of the MIP. The results demonstrated that MIP has higher affinity for the template, Cre over its analogues (Cr and 2-pyr) in single component adsorption. In binary solutions, selectivity of MIP for Cre over 2-pyr was high (3.30 ± 0.34); however, the selectivity over Cr was low (0.46 ± 0.12). In conclusion, Cre-based MIP was successfully synthesized, which is feasible to be used as a diagnostic tool for renal functionality.

CHAPTER ONE

INTRODUCTION

1.1 Molecular imprinting technology

Molecular imprinting technology (MIT) is a rapidly developing method for the preparation of polymers with good selectivity and affinity. MIT often defined as a technique of fabricating a “molecular lock” to pair with a “molecular key”, with the customized binding sites complementary to the target molecules in shape, size and functional group (Chen et al., 2016). Hence, the formation of specific recognition sites is made possible via MIT.

In the imprinting process, the molecular key or the template is allowed to mix with the functional monomers to first form the pre-complex. Next, the pre-complex will undergo polymerization. The template and monomer will be held in position with the presence of cross-linker. The template is then removed from the polymer via extractive solvent, leaving behind cavity with three dimensional structure that is complementary to the target molecule. The interaction between the template and the polymer matrix is similar to that generated from some natural biomolecules to their substrates, such as enzyme-substrate, antibody-antigen as well as receptor-ligand (Wulff, 2002). In other word, the tailored-made binding sites are highly specific which contribute to discrimination ability of the imprinted material.

Generally, there are two main approaches in molecular imprinting for the template-monomer interaction, namely, covalent and non-covalent. These two approaches can be distinguished by the initial formation of the pre-polymerization complex. In covalent approach, the complex is formed via covalent-linkage between the templates and functional monomers prior to polymerization. The rebinding of the

targeted analyte to the imprinted material after template removal, is achieved through the same covalent interactions. This approach has the advantage of strong binding site, nevertheless, it leads to lesser reversibility and lower rate of rebinding as well as removal of template. As for non-covalent approach, template-monomer interactions depend on non-covalent interactions such as hydrogen bonds, ionic interactions, $\pi - \pi$ interactions, hydrophobic forces and Van der Waals attractions (Lee et al., 2013). The association/dissociation kinetics of imprinted polymer via non-covalent approach is normally faster than that observed on that prepared by covalent approach. The benefit of imprinted polymer prepared through non-covalent protocol is that, the removal of template is straightforward via simple diffusion, typically in a polar or acidic solvent that can sufficiently overcome the non-covalent forces between template and polymer (Bergmann and Peppas, 2008). Moreover, non-covalent approach is more flexible regarding the selection of functional monomers and the possible templates. Considering the flexibility and less complicated preparation as compare to covalent approach, non-covalent protocol has been broadly applied.

As a highly crosslinked polymer, the overt advantages given by molecularly imprinted polymers (MIPs) include physical robustness, high strength, and resistance to elevated temperatures and pressures (Li and Li, 2007). Additionally, the imprinted polymers are normally inert to acids, bases, metal ions and organic solvents (Li and Li, 2007). Along with these properties, synthesis of molecularly imprinted polymer (MIP) is rather easy and inexpensive to be carried out. Owing to all the advantages that MIPs have to offer, they are gaining popularity for problem solving in the areas of chemical separations, sensors, and catalysis.

In conclusion, molecular imprinting can be described as a process for the preparation of polymer with the capability of recognizing targeted molecules

selectively. With the evolution of technologies, molecular imprinting will become more significant and will gain acceptance from various field due to the numerous benefits it has to offer.

1.2 Synthesis of molecularly imprinted polymer

There are a few parameters that need to be considered during the synthesis of MIP especially when non-covalent approach is preferred. For instance, template-monomer ratio, monomer-cross-linker ratio, type of porogens as well as polymerization condition (e.g: temperature).

Template-monomer ratio plays an important role in predefining the selectivity of the polymer formed. Too low a template-monomer ratio will result a polymer low cavities and non-specific binding sites, due to over-abundance of functional groups which are scattered randomly throughout the polymer. Likewise, too high ratio will lead to the agglomeration of template besides having insufficient quantity of functional groups to achieve a complete self-assembly, hence a low selectivity polymer (Li and Li, 2007). Cross-linker imparts the mechanical stability to the polymer matrix. Higher cross-linker ratio normally associated with better mechanical stability. However, too high will hinder the diffusion of template both during removal and rebinding process due to the rigid polymer matrix.

Porogen serves as solvent to bring all the components, namely templates, monomers, and cross-linker in one phase. Besides, it helps to create pores in polymers. The properties of porogen like polarity and hydrogen bonding are crucial in defining the final morphology of the polymer structure and porosity. Besides, the stability of the network formed is affected by the elevated temperature during polymerization.

Polymerization at lower temperature is favorable since the process is generally an exothermic reaction. Increase in heat of reaction, as correspond to Le Chatelier's principle, the reaction will favor the reverse reaction.

Sol-gel technique is one of the methods to synthesize molecular imprinted polymer. Catalyst is usually added in the process due to the slow unaided polymerization process. Generally, sol-gel technique uses metal alkoxides as sol-gel precursors since they react readily in water (Brinker and Scherer, 1990). The reaction is known as hydrolysis, as in Eq. (1.1) in Figure 1.1. Hydrolysis may proceed to completion, that is all of the OR groups are replaced by OH, relying on the amount of water and catalyst present (Brinker and Scherer, 1990). Polycondensation takes place after the hydrolysis process to form the polymer matrix. In brief, sol-gel technique is a convenient and flexible method for the preparation of optically transparent, highly stable and porous metal oxide matrices (Lee et al., 2013).

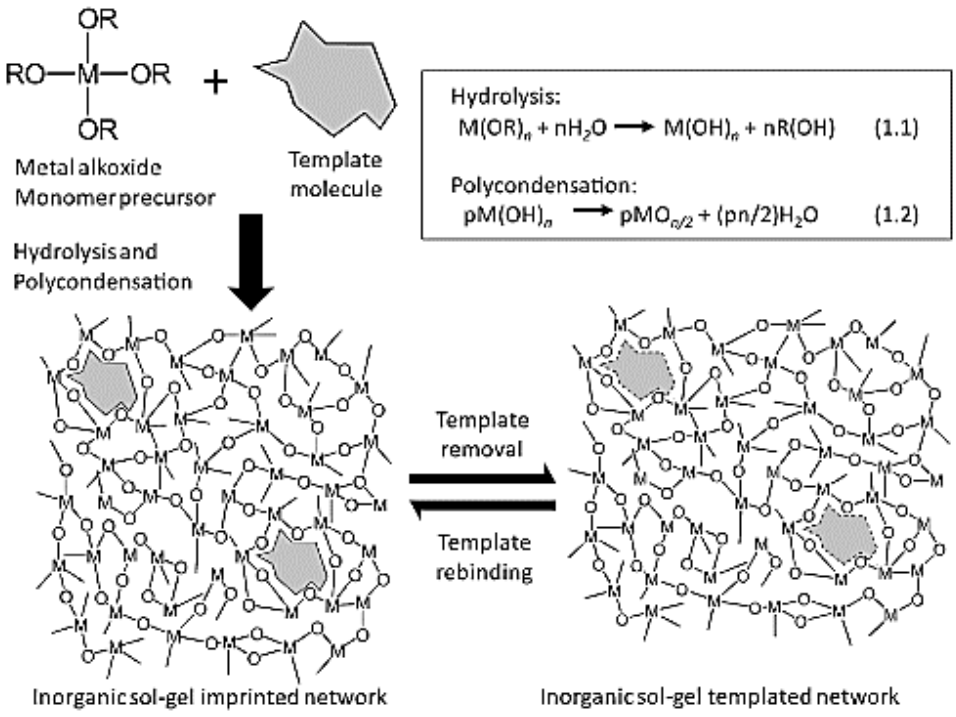


Figure 1.1: Schematic illustration of the molecular imprinting using sol-gel process; R-alkyl radical; M represents a network-forming element such as Si, Ti Zr, Al, etc. (Lee et al., 2013)

1.3 Molecularly imprinted polymer as creatinine sensor

Creatinine is an end product of muscle metabolism, a derivative of muscle creatine phosphate (Fischbach and Dunning, 2009). It is produced continuously in the body and is excreted in the urine. Endogenous creatinine production is constant as long as the muscle mass remains constant (Fischbach and Dunning, 2009). Figure 1.2 shows the role of a kidney in regulating creatinine level in blood serum. Owing to its relatively constant concentration in blood serum and urine, creatinine level serves as a dependable biomarker in assessing renal and muscular function (Ang and Low, 2015).

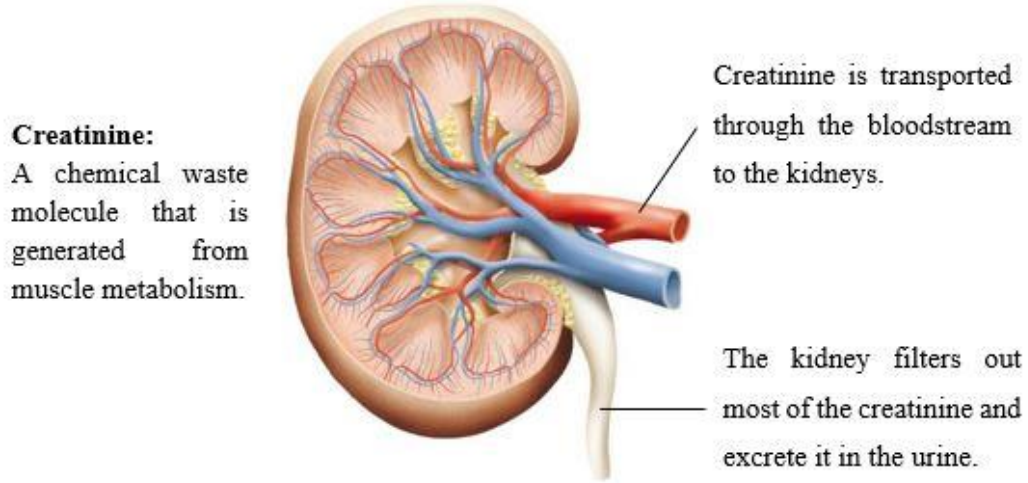


Figure 1.2: Role of a kidney in removal of creatinine (waste) from the body (Nordqvist, 2017).

Analysis methods for the detection of creatinine have been developed since 1886 based on a colorimetric method, which is well-known as Jaffee reaction. (Tsai and Syu, 2011; Ang and Low, 2015). In Jaffe reaction, alkaline sodium picrate is used to react with creatinine and thereby forming a red-yellow complex of Janovsky (Debus et al., 2015). The colored complex is then detected by a spectrophotometric analyzer. However, it is likely to be interfered with and lose its specificity (Ang and Low, 2015). Apart from Jaffe reaction, enzymatic sensors had been reported, though enzymatic

sensor can only work well under limited range of temperature, pressure, and pH. Moreover, enzymatic sensors are often time-consuming and expensive (Tsai and Syu, 2011).

To overcome the limitation of the conventional analysis method for creatinine detection, molecularly imprinted polymer, which offers predetermined selectivity, good mechanical stability over wider temperature and pH range, as well as inexpensive is studied. As interference especially creatine is present in blood serum and urine, the specific recognition of the imprinted polymer produced is an important parameter to be evaluated.

1.4 Problem statement

Creatinine is a waste generated from muscle metabolism which is then carried in the blood stream to kidney. The kidneys maintain the blood creatinine level at normal range. Hence, the serum creatinine concentration is widely used as the indicator for renal function. The conventional method for the measurement of creatinine concentration uses Jaffe's reaction, in which creatinine reacts directly with picrate ion under alkaline conditions to form equimolar red-orange colored complex that can be easily detected and quantified. The major drawback of this method is that in normal subjects as much as 20% of the color reaction in serum arises from substances other than creatinine. This causes the overestimation of creatinine concentration which in turn affects the clinical analysis. In order to improve the analysis method, adsorption method using molecularly imprinted polymer (MIP) is proposed due to its highly specific binding sites towards the targeted molecules.

Certain MIP adsorb better in cold scale while some perform better at higher temperature, depend on the nature of the adsorption process. An adsorption process can either be exothermic or endothermic. The thermodynamic properties such as Gibb's free energy change, enthalpy and entropy are some of the parameters that are critical in estimating the performance and optimization of an adsorption process. For instance, higher temperature is preferable for an endothermic process because it will shift the equilibrium towards the product side, resulting higher rate of adsorption. On the other hand, exothermic process require colder environment for higher rate of adsorption. Thus, adsorption at different temperatures should be conducted to obtain better understanding about the adsorption process of Cre-MIP as well as the suitable temperature for adsorption.

Furthermore, MIP might dissolve in some of the solvents. The solubility of MIP in solvent will affect its adsorption performance. MIP will suffer the loss of its specific recognition sites when it dissolves in the solvent. As a result the ability for the MIP to recognize and adsorb Cre molecules will reduce. Mass loss of the polymer after adsorption is another concern when the polymer dissolves in the solvent during the adsorption test. The mass loss of polymer should be avoided so that the polymer can be regenerate ad reuse for subsequent analyses. Hence, a suitable solvent is needed in order to increase the adsorption capacity of MIP while reducing the mass loss after adsorption.

On top of that, the major disadvantage of the Jaffe's reaction will remain unsolved if specific recognition ability of MIP is not evaluated. Therefore, selectivity tests are worth to be conducted to access the selectivity coefficient of the MIP towards creatinine as well as to prove its reliability in clinical analysis.

1.5 Research objectives

The objectives aligned with this study are as follows:

1. To synthesize and characterize sol-gel-derived silica matrix with imprinted shape cavities of creatinine.
2. To investigate thermodynamic properties of molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP) for better adsorption performances.
3. To select suitable solvent as operating medium for creatinine-based MIP
4. To evaluate discrimination ability of imprinted polymer based on the cavities size, shape and chemical functionality.

1.6 Scope of study

In this study, a molecularly imprinted polymer was prepared through sol-gel process by using creatinine (Cre) as template, tetraethoxysilane (TEOS) as monomer and Al^{3+} as cross-linker. The MIP was synthesized at the template : monomer : cross-linker ratio of 1:5:5. The polymerization was carried out in a water bath of 60℃ for 24 h under constant shaking. The synthesized MIP and NIP were characterized in terms of surface area, and surface area morphology by using Micromeritics ASAP 2020, and SEM respectively.

Thermodynamic studies was conducted to investigate the adsorption behavior of Cre onto MIP. Batch adsorptions were done with 15 mg/L of Cre solution for 24 h with temperature range from 10-50℃. The analyte concentrations before and after the adsorption were measured by UV-vis spectrophotometer at 230 nm.

The effect of adsorption solvent was studied by carrying out the adsorption in different solvents, namely, deionized water, methanol, ethanol and 2-propanol. The experiments were conducted using the initial Cre concentration of 15 mg/L at 30°C for 24 h. The adsorption capacity, imprinting factor as well as the shape factor of MIP were calculated in order to determine the best solvent for Cre adsorption.

Discrimination ability of MIP was evaluated in two ways; ideal selectivity which is adsorption in single component solutions and binary selectivity which is adsorption in two components solutions. Cre, creatine (Cr), and 2-pyrrolidinone (2-pyr) with initial concentration of 15 mg/L were used for this study. The selectivity analysis was performed by a high performance liquid chromatography (HPLC).

1.7 Thesis organization

This work is organized into five chapters, this is the first one: the introduction and objectives of this project. In chapter two, some theoretical concepts are described and explained, for instance the molecular imprinting technology and its applications, polymerization techniques, parameters affecting sol-gel synthesis, as well as development of MIP in renal functionality test. Chapter three presented the materials and methods used during the development of this work. The details of experimental procedures are described in this section. Results and discussion are reported in chapter four. Chapter five contains the conclusions deduced from the present work and recommendations for future work.

In the end, there is a list of references used for this work and appendices that support the accomplished work.

CHAPTER TWO

LITERATURE REVIEW

2.1 Molecular imprinting technology

Molecular imprinting is a technique with predetermined selectivity and high affinity towards recognized species. It is now established as a multifaceted tools for separation, sensors and catalysis technologies. Due to its highly cross-linked structure, molecularly imprinted polymer is essentially stable and potent, aiding its application in extreme conditions such as at high temperature and pressures, in acids or base, and in organic solvent (Haupt and Mosbach, 2000). Furthermore, these materials are cheap to produce and can be stored in the dry state at room temperature over a long period of time (Haupt and Mosbach, 2000).

2.1.1 Principles of molecularly imprinted polymer

The working mechanism of molecularly imprinted polymer (MIP) involved of a synthetic molecular recognition approach based on the "molecular key and lock" principle proposed by Emil Fisher (Lee et al., 2013). Figure 2.1 illustrates the general procedure of molecular imprinting. The templates, which are the "molecular key" are mixed with the functional monomers and allowed to form pre-polymerization complex (Bergmann and Peppas, 2008; Lee et al., 2013). After that, polymerization which can be initiated by certain chemical or physical influence on the system is carried out, where the templates and the monomers are held in position with the aid of cross-linker (Haupt and Mosbach, 2000; Bergmann and Peppas, 2008). Subsequently, the removal of template molecule from the polymer matrix disclose the binding sites that is

equivalent to the size and shape of the template. The most commonly used method for the elimination of template is the washing with extractive solvent (Lee et al., 2013). Throughout template removal, molecule memories are implanted within the polymer matrix which will provide good selectivity and high affinity towards the targeted analytes existed in the testing sample.

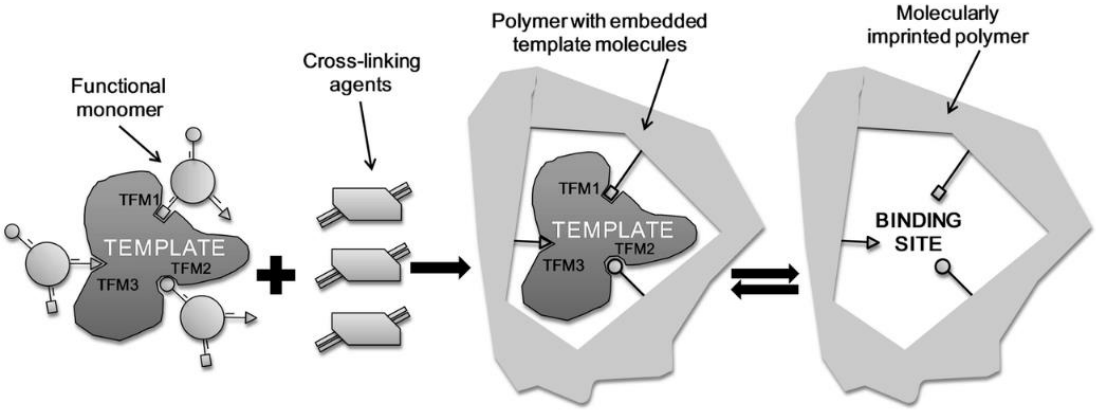


Figure 2.1: Schematic illustration of the molecular imprinting procedure (Lee et al., 2013).

2.1.2 Approaches to molecular imprinting

There are essentially two well-defined approaches to molecular imprinting which are portrayed in Figure 2.2 (Haupt and Mosbach, 2000). These two approaches, namely covalent and non-covalent, are different in the initial formation of the pre-polymerization complex.

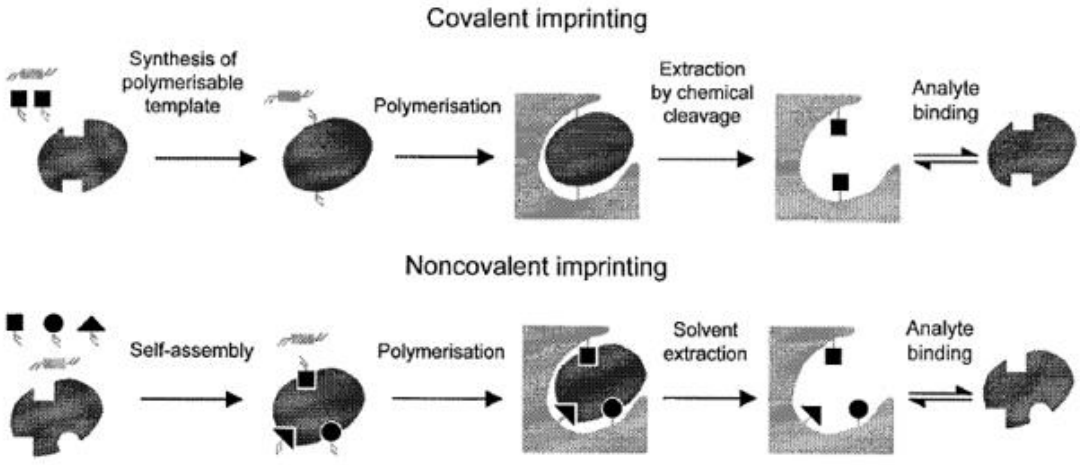


Figure 2.2: Schematic representation of the covalent and non-covalent molecular imprinting procedures (Haupt and Mosbach, 2000).

The covalent approach was first established by Wulff and co-workers (2005). In covalent approach, the template-monomer complex in solution prior to polymerization is restrained by reversible covalent bonds. The imprinted shape of molecule is reliant on the formation of cleavage of these bonds (Yan and Ramström, 2005) Covalent approach has a benefit of strong bindings between template-monomer, rendered to the formation of the rigid imprinted shape. However, the strong covalent interaction has also led to a lesser reversibility and slower speed of the template removal. The covalent linkage owes to be stable under the reaction conditions (polymerization), at the same time it must be easily broken for the removal of template (implanted shape memories within the polymer) (Bergmann and Peppas, 2008). Unfortunately, comparatively few covalent bonds comply these requirements (Bergmann and Peppas, 2008). Consequently, covalent approach is not extensively studied as compare to its non-covalent counterpart.

The non-covalent approach is based on non-covalent interaction (self-assembly) between the template and functional monomers as formulated by Arshady

and Mosbach (1981). For non-covalent imprinting, the most important interactions include van der Waals forces, hydrogen bonding, ionic interactions and hydrophobic forces (Mosbach et al., 2005). This approach has been used widely because of its easy preparation protocol and, less burdensome synthesis of the pre-polymerization complex (Yan and Row, 2006). Besides, the removal of the template is relatively easier via simple diffusion; normally a polar or acidic solvent can adequately overcome the non-covalent interactions between polymer and template (Yan and Row, 2006; Bergmann and Peppas, 2008). Furthermore, the rebinding of the template or detection of the target analyte is much more rapid since the formation of covalent bond is not needed (Bergmann and Peppas, 2008).

2.1.3 Applications of molecularly imprinted polymer

Molecularly imprinted polymer is easy to synthesize, inexpensive and offers improved affinity towards targeted molecule, thus showing a promising potential application in the various fields. A schematic illustration of applications of MIP in different fields is presented in Figure 2.3.

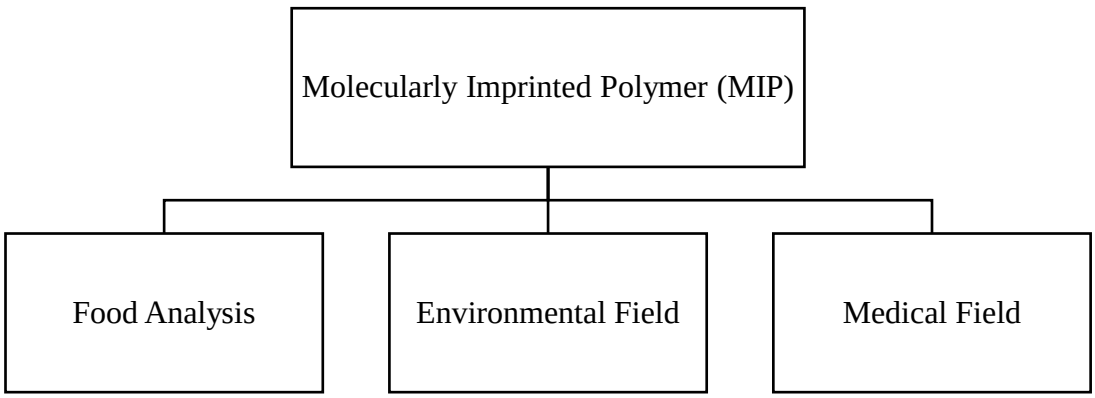


Figure 2.3: Applications of MIP in different fields.

In food analysis, molecularly imprinted polymer is used for a few purposes such as separation or removal of toxic substance, and determination of health beneficial compound (vitamin or mineral) in food sample. The application of molecular imprinting technology for these purposes are studied by a few groups of scientists as list in the Table 2.1:

Table 2.1: Applications of MIP in food analysis.

Applications	**Reference**
Removal of acrylamide (neurotoxic compound) from food sample.	(Ning et al., 2017), (Arabi et al., 2016)
Determination of chlorogenic acid (beneficial compound to human health) in food sample.	(Ribeiro et al., 2016), (Gu et al., 2010)
Detection of amantadine (harmful substance to human health) in animal-derived foods.	(Yun et al., 2017)
Determination of olaquindox (toxic) in food and feedstuffs.	(Wang et al., 2017)
Detection of patulin (toxic) in food.	(Fang et al., 2016)

Reason being, the existing methods, for instance gas chromatography, liquid chromatography, spectrophotometry, and infrared spectrometry are time-consuming, laborious, expensive, and require more complicated instrumentation. Thus, due to its low cost, high selectivity as well as ease of preparation, MIP is gaining its popularity.

Furthermore, molecular imprinting technology has been applied in the environmental field especially for waste management. Removal of lithium ions from wastewater by molecularly imprinting technology has been studied so as to reduce metal pollution to environment as well as to recover lithium for industrial use (Luo et al., 2017). Besides that, antimony which has toxic and carcinogenic properties drew

remarkable attention in environment. A few treatment methods like coagulation, precipitation, coagulation-flocculation-sedimentation and adsorption have been developed. Adsorption method is deemed as an attractive method to remove antimony due to its low cost, simplicity and possible regeneration (Fan et al., 2014). Fan et al. (2014) had developed antimony-imprinted organic-inorganic hybrid sorbents for the removal of antimony due to the good mechanical and thermal stabilities as well as high selectivity that molecular imprinting can offer.

Additionally, molecularly imprinting technology has gained its popularity in medical field. Cholesterol level in human blood serum is to be monitored since abnormal levels in cholesterol leads to several diseases, namely hypertension, and coronary heart disease. Alexander et al. (2017) proposed modified graphene based molecular polymer for cholesterol biosensor. They claimed that enzyme-based sensor is sensitive towards temperature, pH and toxic chemicals. These limitations lead to their attempt to develop a non-enzymatic or so called synthetic sensor. Apart from cholesterol level, the functionality of various human organs are the concerns of all mankind. Kidneys are important organs for human as they function to filter or clean the blood, also to remove wastes and excess fluid from the body. Creatinine level in blood or urine is one of the methods to detect chronic kidney disease (Snyder and Pendergraph, 2005). In order to provide a sensitive and specific detection of creatinine, molecular imprinting technology is adopted because of its specific recognition sites for the targeted molecule. The details will be further discussed in Section 2.5.3.

In short, molecularly imprinted polymer offers high selectivity towards targeted molecule, ease of preparation, low cost, good mechanical and thermal stabilities. Thus, molecular imprinting technology is practical to be applied in numerous fields.

2.2 Polymerization techniques of molecularly imprinted polymer

Imprinted polymer can be prepared via various methods, which include bulk polymerization (He et al., 2007), free radical polymerization (Hilt et al., 2006; Tom et al., 2012; Yu et al., 2013), sol-gel method (Tsai and Syu, 2011), suspension polymerization (Mayes and Mosbach, 1996; Zhang et al., 2003), emulsion polymerization (Yang et al., 2015), and precipitation polymerization (Yusof et al., 2013). Only three methods, namely bulk polymerization, free radical polymerization and sol-gel method will be focused in this report.

2.2.1 Bulk polymerization

Bulk polymerization is one of the simplest techniques that require only monomer and monomer-soluble initiators, possibly a chain-transfer agent for molecular weight control (Carraher, 2010). The general procedure of a bulk polymerization process is illustrated in Figure 2.4. It begins with mixing of functional monomers, cross-linkers, initiators and templates. Porogenic solvent is added if required. Next, the mixture is allowed to undergo polymerization at a fixed temperature for a certain duration of time. Finally, the polymer is washed to remove the templates.

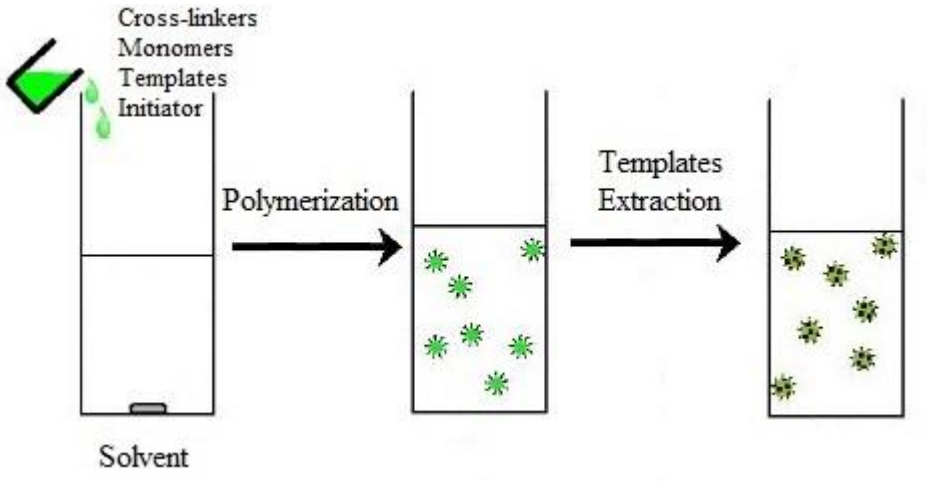


Figure 2.4: Bulk polymerization process.

The rate of bulk polymerization can be observed through the change in volume or increase in viscosity. Since the polymerization is an exothermic process, there is heat buildup. Generally, production in small scale require continuous stirring. Meanwhile, external cooling is desired for the larger scale. The advantages of bulk polymerization includes high polymer yield and easy polymer recovery (Carraher, 2010). However, the limitations are difficulty in removing unreacted monomer and required precise heat control (Carraher, 2010). One of the examples of MIP preparation via bulk polymerization is quinine-imprinted polymer by using methacrylic acid (MAA) as functional monomers (He et al., 2007). He et al. (2007) carried out the polymerization process by two different initiation methods, namely thermal and UV. For thermal initiated polymerization, the process took place in a constant water bath at 55 °C. Meanwhile, the pre-polymerization complex was exposed under ultraviolet lamp (365 nm) at 15 °C for UV initiated polymerization. The details on the effect of polymerization temperature will be further discussed in Section 2.3.5.

2.2.2 Free radical polymerization

Free radical polymerization is a rapid reaction involves series steps includes initiation, propagation, chain transfer, and termination (Figure 2.5). Free radical initiators is used to kick-start the process and is usually at lower levels than the monomer, for instance, 1 wt% or 1 mol% with respect to the total number of moles of monomers (Cormack and Elorza, 2004). The rate and mode of decomposition of an initiator to radical can be regulated in a number of ways, includes heat, light or by chemical (Cormack and Elorza, 2004).

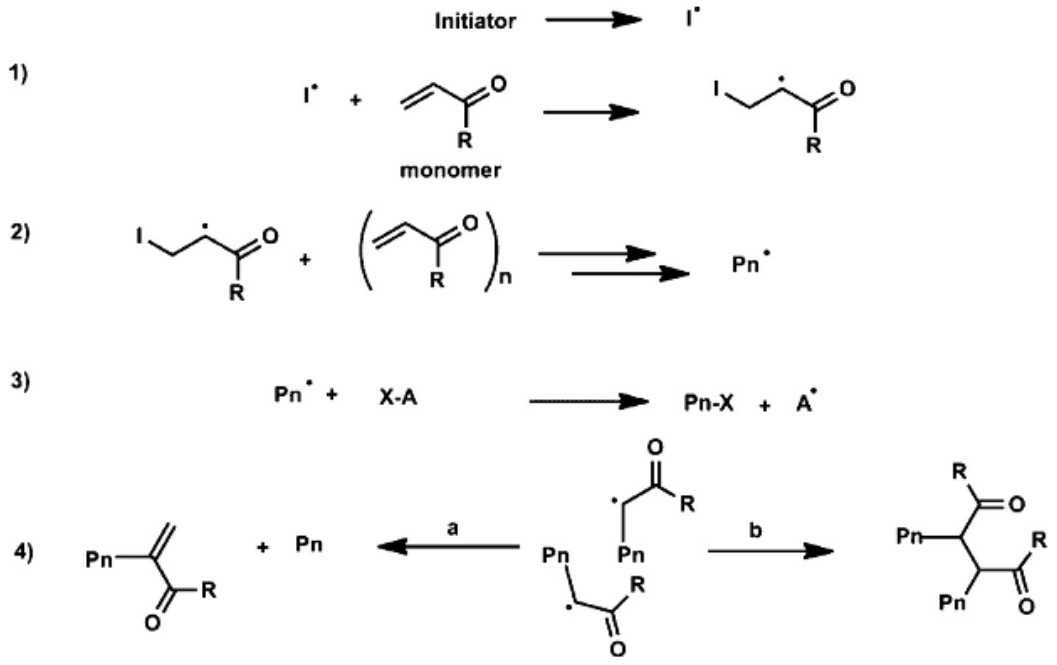


Figure 2.5: Mechanism of free radical polymerization: (1) initiation, (2) propagation, (3) chain transfer, (4) termination via (a) disproportionation and (b) combination (Beyazit et al., 2016).

Free radical polymerizations can be performed under mild reaction conditions, for instance, at room temperature and atmospheric pressure (Cormack and Elorza, 2004). In addition, the vinyl monomers are available commercially at low cost, thus, free radical polymerization is favorable to be used for the preparation of molecularly imprinted polymers (Cormack and Elorza, 2004). Nonetheless, free radical

polymerization hardly offer any degree of control, mainly due to the side reactions that affect the growing species during the polymerization process (Beyazit et al., 2016). Furthermore, the bimolecular termination process and chain transfer reactions are always compete with the propagation steps; hence, result in dead polymer chains with distinct molecular weights (Beyazit et al., 2016).

2.2.3 Sol-gel

The sol-gel process is a useful method of preparing imprinted polymer derived from inorganic monomer, usually metal alkoxides. Imprinted polymer prepared by sol-gel process has become more popular because of its simple procedure at a mild reaction temperature. Generally, metal alkoxides is used as precursors since it is readily to be reacted with water. Hydroxyl ions from water will attach to the metal atom through the hydrolysis process, as described in equation 2.1 (Brinker and Scherer, 1990):

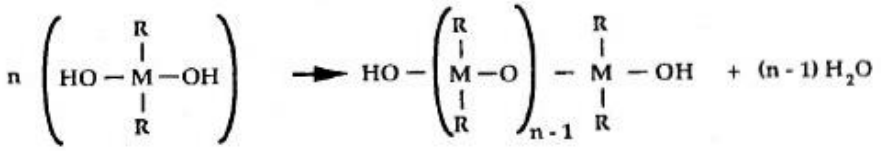
$$M(OR)_4 + H_2O \rightarrow HO - M(OR)_3 + ROH \qquad \text{Eqn. 2.1}$$

R is represented the alkyl group, while M is referred to the metal ions. Hydrolysis may proceed to completion where all the OR groups are substituted by OH group if the amount of water and catalyst is adequate (Brinker and Scherer, 1990):

$$M(OR)_4 + 4H_2O \rightarrow M(OH)_4 + 4ROH \qquad \text{Eqn. 2.2}$$

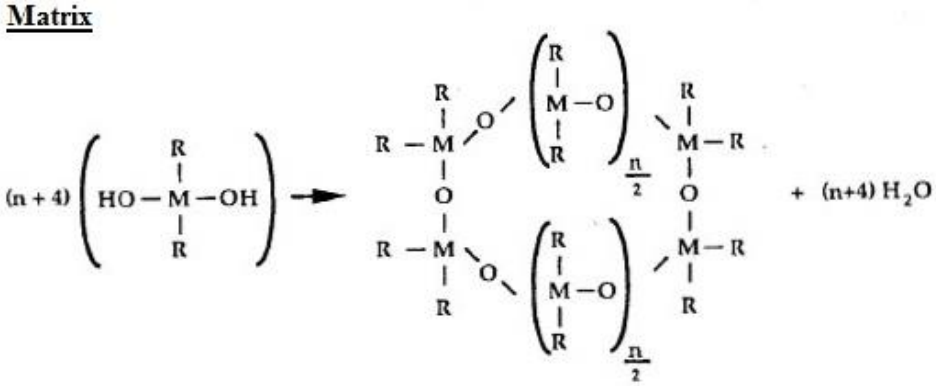
The reaction is then followed by polycondensation for the formation of polymer chain or matrix (Equation 2.3 and 2.4) (Brinker and Scherer, 1990):

Chain



Eqn. 2.3

Matrix



Eqn. 2.4

Sol-gel based molecular imprinting typically requires the use of catalyst since the non-catalyzed polymerization may take weeks to complete (Lee et al., 2013).

The optically transparent, highly stable and porous metal oxide matrices prepared by sol-gel approach at moderate temperatures make it suitable for bio-sensing applications (Lee et al., 2013). Crediting to the superb capability of the entrapment of desired molecules (targeted analyte), sol-gel technology is gaining popularity in the development of the sensing device (Li et al., 2012). Because of the less hazard and simple procedure, the sol-gel method is adopted in this work to synthesize the creatinine-based imprinted polymer.

2.3 Parameters that affect sol-gel synthesis

There are several parameters that affect the sol-gel synthesis of molecularly imprinted polymer namely, template, monomer, cross linker, initiator, porogenic solvent, and polymerization condition.

2.3.1 Template

Template acts as the molecular key in the imprinting process. The success of an imprinted polymer relies on the template-monomer interaction. In general, the template must have chemical groups that can interact with the functional monomers chosen (Bergmann and Peppas, 2008). Besides, the template should be stable under the polymerization conditions (Bergmann and Peppas, 2008). For example, the template should not degrade or should be chemically inert under UV irradiations if UV-initiated polymerizations is carried out. The amount of templates added during synthesis of imprinted polymer affects the success of the polymer formed. Too low of templates added will cause the polymer to have less cavities, nonetheless, too high of templates added will cause agglomeration. Thus, a suitable template to monomer ratio is required to ensure the successfulness of an imprinted polymer.

In a work carried out by He et al. (2007), the selective binding sites increase when the template to monomer ratios vary from 1:3 to 1:5, thus, the adsorption capacity increases. When the template to monomer ratio increases to 1:6, the adsorption capacity reduces, which indicate that excess monomer give rise to non-selective adsorption cavities. Based on their study, template to monomer ratio of 1:5 is the optimum ratio for good adsorption capacity.

2.3.2 Monomer

A monomer is a molecule that combines with other molecules of the same or different type to form a polymer (Rudin and Choi, 2012). Since monomer is the building block of a molecularly imprinted polymer, the amount of monomer added in the polymerization process greatly influenced the specificity of binding sites. Excess monomers in the matrix produce a larger amount of non-specific binding sites hence lowering the selectivity of the imprinted polymer.

Tom et al. (2012) studied the effect of template-monomer ratio; 1:4, 1:6, and 1:15 towards the imprinting factor of sulfadimethoxine (SDM) imprinted polymer. Template-monomer ratio of 1:6 gives the highest imprinting factor, which is 3.94 compare to 1:4 (2.63) and 1:15 (0.89). They inferred that having a slight excess of monomer available in solution during polymerization may optimize the number of interaction between the template and the monomer functional groups which eventually result in imprinting sites; however, having too much excess would either reduces the number of binding sites, or changes the rigidity of the polymer and hence, lessen the cavities left behind by the template molecules as well.

2.3.3 Cross-linker / initiator

The selectivity of a fabricated material is strongly affected by the type and amount of cross-linker used during the synthesis of the material. A cross-linker serves three major functions which includes controlling the morphology of the imprinted matrix, stabilizing the specific binding sites, and bestowing mechanical stability to the imprinted matrix (Yan and Row, 2006). As to obtain perpetually porous materials with competent mechanical stability, high cross-linker ratios are commonly favored

(Cormack and Elorza, 2004). Nevertheless, the amount of cross-linker must not be so high as to restrict diffusion of the template into the network (Bergmann and Peppas, 2008). On the other hand, too little cross-linker produced a larger, less distinguishable cavities since the template pockets will be created too close to each other (Bergmann and Peppas, 2008). Hilt et al. (2006) have investigated the effect of cross-linking percentage towards the selectivity of D-glucose recognized MIP. Polymer networks with a 67% cross-linking percentage has a selectivity of 1.6 towards D-glucose over D-galactose. In search of literature, crosslinking percentage of 80% is often a norm.

Initiator is used in free radical polymerization to react with a monomer to form an intermediate compound (radical) that capable of linking successively with other monomers into a polymeric compound. The commonly used initiators are azo-compounds or peroxides. There are a number of factors that should be considered during the initiator selection, which are the suitability for the use with particular monomers, solvent, and other agents present in the polymerization medium, the types of radicals formed, and the properties the initiator convey to the polymer produced (Moad et al., 2006). For example, under circumstances where complexation is driven by hydrogen bonding and lower polymerization temperatures are favored, photo chemically active initiators will be preferred as these can operate efficiently at low temperature (Cormack and Elorza, 2004).

2.3.4 Solvent

Solvent serves to solubilize all components of the monomer mixture. Apart from that, it acts to create the pores in macro porous polymers, hence it is also known as porogen. A suitable solvent for MIP will produce polymers with well-developed pores high specific surface area (Cormack and Elorza, 2004). An increase in the

solvent concentration will normally lead to an increase in total pore volume (Bergmann and Peppas, 2008). Nonetheless, high solvent concentration induce the formation of microspheres and nanospheres instead of a large and stable crosslinked network (Bergmann and Peppas, 2008).

Furthermore, the selection of solvent must consider the fact that it will not interfere with the template-monomer complex. Song et al. (2009) investigated the effect of porogenic solvent on selective performance of quercetin-imprinted polymer. In their study, quercetin-imprinted polymer was synthesized by thermal polymerization method using quercetin as template, acrylamide as functional monomer and ethylene glycol dimethacrylate as cross-linker in the presence of four different porogenic solvents, namely, 1,4-dioxane, tetrahydrofuran (THF), acetone, and acetonitrile. MIP synthesized in THF exhibited the highest capacity and selectivity. They inferred that the polarity of solvent affects its interaction with template molecule and functional monomer. THF with medium polarity served as the best porogenic solvent among the four solvent tested. Solvent with high polarity like acetonitrile created competition between interactions of template and the functional monomer, and their interaction with porogenic solvent. They deduced that the template-functional monomer interaction is reduced when polarity of porogenic solvent increases.

2.3.5 Polymerization condition

Previous studies revealed that polymerization of MIP at lower temperatures leads to production of polymers with better selectivity as compared to that produced at higher temperatures (Rostamizadeh et al., 2013). Generally, lower temperature is an advantage to the stability of the template–functional monomer assemblies, however,