Effect of the Molecularly Imprinted Polymer Component Ratio on Analytical Performance

Kelvin Fernando Pratama, Maretty Erwanta Roulina Manik, Driyanti Rahayu, and Aliya Nur Hasanah*

Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University; Jl. Raya Bandung Sumedang KM 21.5 Jatinangor, Sumedang 45363, Indonesia.
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Molecular imprinting technology is a new analytical method that is highly selective and specific for certain analytes in artificial receptor design. The renewal possibilities of this technology make it an ideal material for sundry application fields. Molecularly imprinted polymers (MIPs) are polymeric matrices that have molecules printed on their surfaces; these surfaces can chemically interact with molecules or follow the pattern of the available template cavities obtained using imprinting technology. A MIP is useful for separating and analysing complex samples, such as biological fluids and environmental samples, because it is a strong analytical recognition element that can mimic natural recognition entities like biological receptors and antibodies. The MIP components consist of the target template, functional monomer, crosslinker, polymerisation initiator, and porogen. The effectiveness and selectivity of a MIP are greatly influenced by variations in the components. This review will provide an overview of the effect of MIP component ratio on analytical performance to each target analyte; it will also provide a strategy to obtain the best MIP performance. For every MIP, each template: monomer: crosslinker ratio shows a distinct performance for a specific analyte. The effects of the template: monomer: crosslinker ratio on a MIP’s analytical performances—measured by the imprinting factor, sorbent binding capacity, and sorbent selectivity—are briefly outlined.

Key words  molecularly imprinted polymer component; imprinting factor; sorbent selectivity; molecularly imprinted polymer analytical performance; sorbent binding capacity

1. Introduction

Nowadays, the idea of molecular imprinting has been broadly perceived as the most advanced technology for preparing various materials that have a specific recognition site with selective adsorption. This technique has generated much attention from the scientific community over the past years.1,2) The most widely used implementation of this technique is the synthesis of molecularly imprinted polymers (MIPs). MIPs are three-dimensional polymers with specific recognition sites for a certain molecule. A MIP is formed by crosslinking template with monomers to create copolymers.3–5) A MIP is an engraved material; a template creates specific cavities that recognise molecules with the same size, shape, and functional groups.3,6) The main benefits of a MIP are the high selectivity and affinity for the specific molecule used in the imprinting procedure, the relatively inexpensive synthesis, and longer degradation times that make their recognition sites last for quite a while at room temperature.5,7) Due to these advantages, MIPs have attracted extensive interest and have been applied in numerous areas, including biomolecules, and are still being studied for further application for other processing, such as sensors, chromatography, catalysis, separation science, and solid-phase extraction.5,7)

Following the literature reports published over the decade, MIP has been widely applied in variety areas. In solventless extraction techniques, MIPs use as a selective sorbent in solid-phase extraction (SPE) (MI-SPE) and considered as the most advanced application of MIP materials because it could almost completely eliminate interfere in matrix samples.8) MIP materials also employed as a selective sorption medium in solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE), and matrix solid-phase dispersion membranes (SLM).9–11) In chromatographic separation techniques, MIP applied as a stationary phase that could selectively recognize chiral molecules.12,13) In chemical sensing, MIP uses as a recognition layer that is sensitive to chemical changes in the surrounding environment.14) One of MIP sensors was developed by Guerreiro et al.15) to develop electrochemical mercury sensors constructed with a methylene blue (MB)-modified and thymine-containing linear DNA probe. Similarly, Liu et al.16) reported a piezoelectric sensors for detection and discrimination of volatile carboxylic acid components.

MIP performance is analysed by the polymer’s affinity for a specific analyte, the specificity of imprinted materials, and interaction(s) in the binding sites, which is measured by its separation.17) This performance is influenced by several factors: functional monomer, crosslinking agent, porogen, polymerisation method, polymerisation initiator, type of solvent,
time, and temperature of the polymerisation process, among others.\textsuperscript{18} The suitability ratio of an MIP formulation is fundamental to maintain the stability of the cavity and the polymer matrix. Using a large amount of monomer for MIP synthesis should increase non-covalent interactions in the polymerisation process and affect the adsorption ability; this phenomenon would alter the imprinting factor (IF).\textsuperscript{19} Yilmaz et al.\textsuperscript{19} illustrated that a lower amount of functional monomer compared to cross-linking agent can depress number of possible high-quality binding sites, with consequent lower IF values. Andersson et al.\textsuperscript{20} and Tom et al.\textsuperscript{21} showed that lower amount of template compared to its functional monomer results in higher binding efficiency due to the increase of homogenous binding sites. Yoshimatsu et al.\textsuperscript{22} however, determined that increasing the amount of functional monomer decreased the binding capacity, and thus reduced rigidity and selectivity of the polymer.

The selection of proper components is critical for the synthesis of a MIP with desirable characteristics. Indeed, carefully choosing the components is the most important factor to optimise a MIP’s affinity for a specific analyte. The quality of the interactions between template and functional monomer influences a MIP’s affinity and thus determines its properties, such as the accuracy and selectivity in the recognition sites.\textsuperscript{23}\textsuperscript{25} Furthermore, the ratios among functional monomer, template, and crosslinking agent also influence a MIP’s affinity and imprinting efficiency.

Although MIPs have been extensively studied, a summary of how a MIP’s composition affects its analytical performances has only been examined in a few articles. In this review, we offer suggestions on how to successfully attain the most suitable conditions for the synthesis of a high-performance MIP. First, we present a brief overview of the principal components of a MIP, including target templates, functional monomers, crosslinkers, porogens, and initiators. Second, we highlight the effect of the MIP composition ratio on its experimental parameters, namely IF, sorbent capacity, and sorbent selectivity. We hope this review provides an overview of how to choose the appropriate composition to synthesise a MIP for specific analytical interests. We expect that this review will open further research into the discovery and development of MIP related to the composition ratio of MIP performances.

2. Essential Components of a MIP

MIP components comprise a template, a functional monomer, a crosslinker, a polymerisation initiator, and a solvent. MIP preparation is highly dependent on the features of each of its components, such as the type and amount of monomer, amount of crosslinkers, type of initiator, solvent polarity, temperature, and the polymerisation process.\textsuperscript{18,24}

2.1. Target Templates

The template has an important role in organising functional groups into functional monomers. The functional group of a template, such as amino, carboxyl, hydroxyl, amide, and ester, plays an important role in MIP performance.\textsuperscript{23}\textsuperscript{25} Highly polar groups would make it easier to prepare a high-performance MIP because more stable molecular complexes are formed. Polymers that form hydrogen bonds with functional monomers may have high selectivity and affinity because of the contribution of hydrogen bonds to directional, saturation, and strength.\textsuperscript{26}

Molecular imprinting aims to synthesise a MIP that is similar to a biological receptor; hence, it could be applied to replace such a biological entity. There are three requirements for an ideal template: first, its functional groups do not inhibit polymerisation; second, it shows outstanding chemical stability in the polymerisation process; and third, its functional groups can construct complexes with functional monomers.\textsuperscript{27} Target templates in many areas, such as environmental, biological, pharmaceutical, chemical, industrial, and clinical interests, have been broadly used for MIP synthesis. The imprinting of organic molecules (e.g. atrazine, tetracycline, tyrosine) and ion-imprinted polymers for several kinds of toxic heavy metals (e.g. aluminium ion, mercury ion, chromium ion) has been well established. Biological macromolecules such as proteins or viruses also can be imprinted through similar approaches, but the imprinting technique for this target template is still a challenge.\textsuperscript{24,25} Macromolecules such as proteins are extremely complex and have non-specific recognition sites at their surfaces due to the presence of charged residues. Verheyen et al.\textsuperscript{28} used lysozyme as the template; they showed non-specific electrostatic interactions between lysozyme and positively and negatively charged networks. A high molecular weight retards a protein’s diffusion through the dense polymer network; this factor makes synthesis of protein-imprinted polymer challenging. Hawkins et al.\textsuperscript{29} synthesised HydroMIP for Fluorescein Isothiocyanate (FITC)-albumin recognition; they showed a MIP with a low percent recovery (only 57.3%). Thus, several methods have been established and optimised for extraction of protein as a template. In line with the previous study, Ou et al.\textsuperscript{30} produced an imprinted polymer with lysozyme as the target template that neither efficiently separated proteins with similar molecular weight and isoelectric point (such as cytochrome c) nor proteins with marked differences in dimensions and charge (such as albumin).

2.2. Functional Monomers

Functional monomers have a role in forming pre-polymerisation complexes with templates. The functional groups of functional monomers will interact and bind to the template molecule. In this case, it is important to choose an applicable functional monomer that can sturdily interact with the template to form the specific donor–receptor or antibody–antigen complexes before the polymerisation process.\textsuperscript{24} The selection of the right functional monomer is essential to produce specific cavities designed as template molecules.\textsuperscript{31}

The total amount of functional monomers used in generating a MIP is finite; it can restrict the selectivity and further application of a MIP. In forming a strong interaction with the template, it is crucial to design and integrate a new functional monomer that has high selectivity and specificity to the target analyte so that it can interact with the template. There are two common sites in a functional monomer: one for recognition and one for polymerisation. The mechanisms and interactions between the functional monomer and template molecule occur in the pre-polymerisation process, which is determined by the quality and quantity of the recognition unit of a MIP.\textsuperscript{24,31}

There are several types of functional monomers, carboxylic acid, sulphonamic acid, heteroaromatic, aniline, and pyrrole, among many others. Examples of carboxylic acid type monomer are methacrylic acid (MAA), acrylic acid, and benzoic acid. Besides, sulphonamic acid monomers include acrylamide methylpropane sulphonamic acid, while heteroaromatic base monomers are vinylpyridine and vinylimidazole.\textsuperscript{2,22} Huang
et al. \cite{33} used MAA for progesterone recognition. Banerji et al. \cite{34} synthesised a MIP for glucose detection with polyallylamine as a functional monomer. Matsui et al. \cite{35} generated a MIP for dopamine with acrylic acid-N-isopropyl acrylamide as the functional monomer. Some typical functional monomers are shown in Fig. 1.

Some researchers have utilised other functional monomers. For example, the detection of $\beta$-lactam antibiotics has incorporated polymerisable amidines and ureas that have been expanded to become a polymer receptor. \cite{37} In addition, poly[(2-oxo-1, 3-dioxolan-4-YL) methyl methacrylate-co-acrylonitrile] monomer and polyamide-imide polymer have been assessed for the arrangement of a surface plasmon resonance (SPR)-sensitive film sensor. \cite{38} Zhang et al. \cite{39} synthesised a water-soluble cross-linker double as functional monomer for naproxen sodium imprinted polymers. Besides, some researchers have used ionic liquids (ILs) as monomers. Polymerisable ionic fluids (PILs) are typically composed of an IL monomer; imidazolium-cation type ILs are most often used for a direct polymerisation process. There are numerous IL monomers (cations and anions) that have been made also applied to prepare PILs. \cite{40,41} Sardar et al. \cite{42} synthesised monomer from the IL 1-(6-hydroxyhexyl)-3-methylimidazolium bromide, which is a potent functional group from methacrylate for the IL backbone, a component for next the polymerisation step.

2.3. Crosslinkers

A crosslinker functions to set the functional monomers over template molecules in the polymerisation process. Using such a component in MIP production will generate a very crosslinked rigid polymer after the template is removed. The selectivity and binding capacity of a MIP is influenced by the type and number of utilised crosslinkers. \cite{33} Normally, if too little crosslinker is used, the MIP will be mechanically unstable. On the other hand, the number of recognition sites in a MIP decreases when higher amounts of crosslinker are employed. \cite{24}

A MIP preparation exploits covalent and non-covalent interactions. The specific interaction is formed between the template and functional monomer in the solution with an analyte. \cite{32,44} The method of preparing a MIP with covalent imprinting assures that the imprinted cavity has a functional monomer residue and is stoichiometric. Covalent imprinting involves polymer binding that depends on reversible covalent bonds. MIP preparation with covalent imprinting uses a crosslinker such as triallylic isocyanurate (TAIC), bis-(1-(tert-butylperoxy)-1-methylethyl)-benzene (BIPB), or dicumyloveroxide (DCP). By contrast, non-covalent imprinting involves ionic interactions, hydrogen bonds, Van der Waals, and $\pi-\pi$ interactions. Hydrogen bonds are the most common interaction. Non-covalent imprinting methods have become more extensively used. MIP preparation with non-covalent imprinting uses a crosslinker such as triallylic isocyanurate (TAIC), bis-(1-(tert-butylperoxy)-1-methylethyl)-benzene (BIPB), or dicumyloveroxide (DCP). By contrast, non-covalent imprinting involves ionic interactions, hydrogen bonds, Van der Waals, and $\pi-\pi$ interactions. Hydrogen bonds are the most common interaction. Non-covalent imprinting methods have become more extensively used. MIP preparation with non-covalent imprinting uses a crosslinker such as triallylic isocyanurate (TAIC), bis-(1-(tert-butylperoxy)-1-methylethyl)-benzene (BIPB), or dicumyloveroxide (DCP).

2.4. Porogen (Porogenic Solvent)

Porogens are used for dispersing media and pore-forming agents in the polymerisation process. A porogen dissolves all components used in the MIP synthesis process. \cite{45} A porogen’s polarity can affect interactions between the template and functional monomers. It affects the adsorption properties of a MIP, especially in non-covalent interaction systems. For a non-covalent imprinting method, a non-polar or less polar organic porogen, such as toluene, acetonitrile, or chloroform, is used to obtain good printing efficiency. The type of porogen or solvent used will determine the adsorption and morphological properties of the formed polymer. \cite{24}

2.5. Initiators

An initiator is a compound that will trigger the initiation of polymer chains and accelerate poly-
merisation. The number of employed initiators is considerably less than the number of functional monomers. The processes to prepare a MIP include free radical polymerisation (FRP), photopolymerisation, and electropolymerisation. FRP can be started either thermally or photochemically for a wide range of functional groups and template structures. There are several types of initiators used in the process of MIP preparation; azobisisobutyronitrile (AIBN), azobisdimethylvaleronitrile (ADVN), benzoyl peroxide (BPO), dimethyl acetal of benzyl (BDK), and potassium persulfate (KPS) are the most common.

Photopolymerisation is a type of FRP where the process is initiated with light. There are several advantages to this process: reduced energy consumption, higher productivity, less waste, and a lower reaction temperature. The limitation of photopolymerisation is a restriction in the depth of light penetration; this factor will depend on wavelength and spectral distribution. Subjecting the relevant photoinitiators to UV irradiation will create the photochemical initiation process. Generally, photopolymerisation uses organic photoinitiators. In determining a particular type of function, organic polymerisation photoinitiators are divided into various classes. In order to obtain the best total cure, fast speed, and a minimal colour formation, a mixed type of photoinitiator is often used. Acetophenone, benzyl and benzoin compounds, benzo-phenone, miscellaneous, thioxanthones, and cationic photoinitiators are the most commonly used organic photoinitiators.

Electropolymerisation is an initiator process that controls the number of cycles or currents applied to the electrode to achieve a polymer film of a particular thickness. An electro-synthesised polymer could be used as the matrix to support MIP particles and establish their contact to the electronic surface. For this purpose, MIP particles are suspended in monomer solution, followed by polymerisation. Electropolymerisation is an advantageous technique for MIP preparation based electrochemical sensor. Choong and Milne synthesised MIP chemosensors by electropolymerisation and applied potential pulses between 0.3 V (1 s) and 0.7 V (10 s) against saturated calomel electrode (SCE). The pattern formed on the surface of the vesicles–polydiacetylene with coordinated arrays of receptor site—is caused by the presence of light-induced polymerisation.

3. Analytical Performance of a MIP in Terms of the Polymer Component Ratio

Molecular imprinting synthesises a polymer that is specific for a certain molecule due to its recognition sites. This technique has been applied in many areas, such as a recognition element for immunoassays, sensors, and separation (e.g., for chromatographic and solid-phase extraction media). A MIP is an excellent choice to achieve high selectivity in extractions due to its ability to reduce interferences and matrix effect in the polymerisation process that develop selective and precise analytical methods through increasing chromatographic separation and detection. MIPs have been widely used in several adsorptive phase in solid-phase extraction (SPE), dispersive solid-phase extraction (DSPE), solid-phase microextraction (SPME), and stir bar sorptive extraction (SBSE). It has been recently applied to extract various analytes from complex samples. The use of MIP in SPE as a sorbent can enhance selectivity, sensitivity, and reproducibility. Molecularly imprinted solid-phase extraction (MI-SPE) is the most advanced application of a MIP for selective extractions or to clean up target analytes from different kinds of samples. MI-SPE is commonly coupled with HPLC or GC to determine targeted analytes. Capillary electrophoresis (CE) is also a common tool considering its high resolution, fast separation, and small sample and reagent consumption.

Notably, advanced applications of a MIP depend on the development of materials with higher performance. The IF, sorbent binding capacity, and sorbent selectivity are important to evaluate a MIP’s performance. The quality and performance of the synthesised polymer depends on the characteristic of the target template, functional monomers, and
crosslinker(s) that create functional complementarity between the target molecule and molecular recognition sites. This review presents a reasonable approach to improve the performance of MIPs.

The specific component ratio employed to synthesise a MIP greatly affects the physical and chemical properties of MIP. These features, in turn, will impact its analytical performances in sample separation and recognition site and will also determine a MIP’s application(s). The effect of a MIP’s component ratio on its experimental parameters, such as the IF, sorbent binding capacity, and sorbent selectivity summary, are presented in Table 1. These factors will influence a MIP’s analytical performance, namely the recovery percentage of the MIP towards the analyte and selectivity of the analyte to compare to the analog compound. The interaction mode is also very important key factor to prepare the proper MIPs. The interaction mode between template and functional monomers, are presented in Table 2.

3.1. IF The IF is one of the important factors used to assess the performance of a MIP. The IF indicates the strength of the polymer interactions that occur against the template molecule. The IF involves the specific recognition properties of a MIP and non-imprinted polymer (NIP) regarding a specific template. The IF value is calculated from the retention factor (k’) ratio of MIP with NIP, using the formula:

$$IF = \frac{k’_{MIP}}{k’_{NIP}},$$

where $k’_{MIP}$ indicated the MIP retention factor and $k’_{NIP}$ indicates the NIP retention factor.

There is no maximum value for the IF, but it must be greater than 1. An IF of 1 implies that there is no difference in analyte recognition between the MIP and the NIP. If the value is greater than 1, then the MIP is better than the NIP. The IF can be used to determine and distinguish the interactions formed between a MIP and an analyte. The IF indicates the molecular bond(s) that occurs between a MIP and an analyte rather than the physical bond(s). A NIP can help determine correct interactions due to molecular recognition. The molecular interaction formed between a MIP and an analyte relies on binding between the functional group and functional monomer selectivity. The IF value can interpret MIP performance in terms of separation analysis because it is the value of adsorption capacity for a template bound to a MIP. In addition to its use as a pre-treatment technique to indicate high selectivity and affinity for specific analytes, a MIP has several other uses. It is commonly employed in HPLC as the stationary phase, and it can be used in capillary electrochromatography, capillary LC, and TLC as the packing and column materials.

Some research results have shown that the IF is influenced by the MIP component ratio. Liu et al. synthesised a MIP for ciprofloxacin (CIP) recognition; they prepared it with bulk polymerisation. In this MIP preparation, they used CIP as the template molecule, MAA as a functional monomer, and TRIM as a crosslinker. The carboxyl and –NH functional groups on the piperidic ring of CIP molecule forms non-covalent interaction with carboxyl of MAA. The IF was 1.37 when they optimised the template: monomer: crosslinker ratio as 1:6:16. On the other hand, Jungang et al. synthesised a MIP with the same materials and method; their IF was 2.28 with an optimised template: monomer: crosslinker ratio of 1:6:20. Liu et al. explained that less crosslinker will create long, free polymer chains within large pore diameters and low mass transfer resistance due to fewer crosslinking sites. The polymer aggregation morphology would be altered after elution and the initial shape and size of MIP cavities would not be maintained by the polymer. These changes led to poor CIP binding properties. The polymer rigidity and expansion were altered in different solvents and enhanced by the increased number of crosslinker. Jungang et al. showed the elevated IF led to good recognition selectivity for CIP: the separation factor ($a$) for the MIP ($a = 2.03$) was much higher than that of the NIP ($a = 0.63$). This MIP can be applied as the sorbent for solid-phase extraction to recognise and separate CIP in subsequently analysis.

Song et al. synthesised MIPs for erythromycin separation. These MIPs employed SPE and non-covalent bulk polymerisation. They used MAA as the functional monomer and EDGMA as the crosslinker. Erythromycin has five hydroxyl groups and a tertiary amine on one of the sugar units which can form a hydrogen bond and an ionic bond with MAA. The template: monomer: crosslinker ratios were 1:2:20 (MIP1), 1:4:20 (MIP2), and 1:8:20 (MIP3). MIP1 had the best performance based on its binding to erythromycin, with an IF of 1.70. The MIP2 and MIP3 ratios showed decreased affinity and smaller IF values (1.35 and 1.11, respectively). Song et al. illustrated the specific absorption of the MIPs decreased by degrees when the functional monomer:template ratio increased from 2:1 to 8:1. If there is too much monomer, there will be increased non-specific absorption in the MIPs. The specific absorption of MIPs was modified with the ideal amount of template to monomer. The optimised MIP-SPE revealed that MIP1 recognises erythromycin with average cross-reactivity to other macrolide antibiotics and high selectivity for erythromycin, with a recovery of around 89%. These MIPs can be used in biological and environmental field analysis applications.

Hasanah et al. synthesised and characterised five MIPs for glibenclamide separation. They used a sorbent for SPE and prepared MIPs with different component ratios. They used glibenclamide as the template, acrylamide as the functional monomer, and EDGMA for the crosslinker. Acrylamide has amine and carbonyl functional groups that can bind to the carbonyl and amine group of glibenclamide to form hydrogen bonds. The template: monomer: crosslinker ratios were 1:6:40 (MIP1), 1:6:60 (MIP2), 1:6:70 (MIP3), 1:4:40 (MIP4), and 1:15:40 (MIP5). The IFs were: MIP1 (3.22), MIP2 (1.63), MIP3 (6.85), MIP4 (1.33), and MIP5 (1.11). Based on the results, MIP3 had the highest IF; this MIP had a higher monomer:template ratio (6:1) with a crosslinker ratio of 70. These results could be because there was an adequate amount of acrylamide that favoured the maximum interaction between glibenclamide and acrylamide and enhanced quality of the imprinted sites in the polymer. MIP3 also had a high amount of crosslinker (70), a factor that creates the rigid polymer and maximises the integrity of binding site. Besides, MIP3 could bind up to 88.81% to glibenclamide in acetoniitrile solution (pH 4) compared to NIP3, which only bound up to 54.34%. This result illustrated that a higher monomer amount will increase the binding ability due to greater non-covalent interactions. MIP3 might be useful for SPE when purifying and concentrating glibenclamide, although it requires further optimisation.
| Analyte              | T<sup>a</sup> | fM<sup>a</sup> | X<sup>a</sup> | Initiator | Porogen             | T : fM : X ratios | Method                        | Analytical performances | Application                                                                 |
|---------------------|--------------|--------------|-------------|-----------|----------------------|-------------------|-------------------------------|--------------------------|----------------------------------------------------------------------------|
| Ciprofloxacin       | Ciprofloxacin| MAA<sup>a</sup> | TRIM<sup>a</sup> | AIBN<sup>a</sup> | Methylbenzene        | 1 : 6 : 16        | Bulk polymerisation            | 1.37                     | —                           | 41.64 mL/g               |
|                     |              |              |             |           | Toluene              | 1 : 6 : 20        |                               | 2.28                     | 0.81 mL/g                  |
| Erythromycin        | Erythromycin | MAA          | EGDMA<sup>a</sup> | AIBN | Acetonitrile/methanol | 1 : 2 : 20        | Bulk polymerisation            | 1.70                     | 89%                        | 0.83 mg/mL                |
|                     |              |              |             |           | 1 : 4 : 20           |                   |                               | 1.35                     | —                          | —                         |
|                     |              |              |             |           | 1 : 8 : 20           |                   |                               | 1.11                     | —                          | —                         |
| Glibenclamide       | Glibenclamide| AAm<sup>a</sup>| EGDMA      | AIBN | Chloroform           | 1 : 6 : 40        | Bulk polymerisation            | 3.22                     | —                          | —                         |
|                     |              |              |             |           | 1 : 6 : 60           |                   |                               | 1.63                     | 89%                        | 0.83 mg/mL                |
|                     |              |              |             |           | 1 : 6 : 70           |                   |                               | 6.85                     | —                          | —                         |
|                     |              |              |             |           | 1 : 4 : 40           |                   |                               | 1.33                     | —                          | —                         |
|                     |              |              |             |           | 1 : 15 : 40          |                   |                               | 1.11                     | —                          | —                         |
| Cinnamic acid       | Cinnamic acid| AAe<sup>a</sup>| DVB<sup>a</sup> | AIBN | Acetonitrile         | 1 : 6 : 20        | Precipitation polymerisation  | 3.842                    | 80.7%                      | —                         |
|                     |              |              |             |           | 1 : 4 : 20           |                   |                               | 2.91                     | 61.3%                      | —                         |
|                     |              |              |             |           | 1 : 6 : 28           |                   |                               | 2.44                     | 51.3%                      | —                         |
| Pseudoephedrine     | MAA          | EGDMA        | AIBN       | Chloroform | 1 : 2 : 63          | Precipitation polymerisation | 20                     | —                       | 3960 mL/g             |
|                     |              |              |             |           | 1 : 3 : 63           |                   |                               | 9                       | —                         | —                         |
|                     |              |              |             |           | 1 : 4 : 63           |                   |                               | 1.152                    | —                          | —                         |
|                     |              |              |             |           | 1 : 6 : 63           |                   |                               | 0.921                    | —                          | —                         |
|                     |              |              |             |           | 1 : 8 : 63           |                   |                               | 1.02                     | —                          | —                         |
| Piperine            | Piperine     | AAe          | EGDMA      | AIBN | Acetonitrile         | 1 : 4 : 16        | Precipitation polymerisation  | —                       | 69.40%                    | —                         |
|                     |              |              |             |           | 1 : 4 : 24           |                   |                               | —                       | 75.86%                    | —                         |
|                     |              |              |             |           | 1 : 6 : 16           |                   |                               | 2.86                     | 84.94%                    | 76.72                     |
|                     |              |              |             |           | 1 : 6 : 24           |                   |                               | —                       | 60.80%                    | —                         |
| 2-Phenylphenol      | 2-Phenylphenol | Styrene | DVB        | AIBN | Acetonitrile         | 1 : 2 : 16        | Precipitation polymerisation  | 2.04                     | —                          | —                         |
|                     |              |              |             |           | 1 : 3 : 16           |                   |                               | 1.87                     | —                          | —                         |
|                     |              |              |             |           | 1 : 4 : 16           |                   |                               | 2.14                     | 99.52%                    | 90                        |

<sup>a</sup> T, fM, and X represent the ratios of template, monomer, and crosslinker, respectively.

<sup>1)</sup> Not mention their specific application in the article, but could be used for any application which needed a sorbent to separate target analyte.

<sup>2)</sup> Used as solid-phase extractants in animal-origin foods and analysis in biological samples.

<sup>3)</sup> Used as solid-phase extractants in animal-origin foods and analysis in biological samples.

<sup>4)</sup> Not mention their specific application in the article, but could be used for any application which needed a sorbent to separate target analyte.

<sup>5)</sup> Used as SPE, column packing materials, and for drug delivery system.

<sup>6)</sup> Used in medical analysis of biological fluids.

<sup>7)</sup> Not mention their specific application in the article, but could be used for any application which needed a sorbent to separate target analyte.

<sup>8)</sup> Analysis in biological and environmental field.

References:

1. Ciprofloxacin Ciprofloxacin MAA TRIM AIBN Methylbenzene 1 : 6 : 16 Bulk polymerisation 1.37 — 41.64 mL/g Not mention their specific application in the article, but could be used for any application which needed a sorbent to separate target analyte.

2. Erythromycin Erythromycin MAA EGDMA AIBN Acetonitrile/methanol 1 : 2 : 20 Bulk polymerisation 1.70 89% 0.83 mg/mL Used as solid-phase extractants in animal-origin foods and analysis in biological samples.

3. Glibenclamide Glibenclamide AAm EGDMA AIBN Chloroform 1 : 6 : 40 Bulk polymerisation 3.22 — — Not mention their specific application in the article, but could be used for any application which needed a sorbent to separate target analyte.

4. Cinnamic acid Cinnamic acid AAe DVB AIBN Acetonitrile 1 : 6 : 20 Precipitation polymerisation 3.842 80.7% — Used as SPE, column packing materials, and for drug delivery system.

5. Pseudoephedrine Pseudoephedrine MAA EGDMA AIBN Chloroform 1 : 2 : 63 Precipitation polymerisation 20 — 3960 mL/g Used in medical analysis of biological fluids.

6. Piperine Piperine AAe EGDMA AIBN Acetonitrile 1 : 4 : 16 Precipitation polymerisation — 69.40% — Not mention their specific application in the article, but could be used for any application which needed a sorbent to separate target analyte.

7. 2-Phenylphenol 2-Phenylphenol Styrene DVB AIBN Acetonitrile 1 : 2 : 16 Precipitation polymerisation 2.04 — — Analysis in biological and environmental field.
Table 1. Effect of Molecularly Imprinted Polymer (MIP) Component Ratio on Its Analytical Performances

| Analyte          | T | fM | X | Initiator | Porogen | T : fM : X ratios | Method               | Analytical performances | Application References |
|------------------|---|----|---|-----------|---------|------------------|-----------------------|-------------------------|-------------------------|
| Congo red        | Congo red | MAA | EGDMA | AIBN | Acetonitrile | 0.1 : 4 : 20 | Precipitation polymerisation | 2.80 | 97.12% | 79 | Analysis in environmental field |
|                   |     |     |     |       |          | 0.1 : 6 : 20 |                         | 2.68 | — | — | |
|                   |     |     |     |       |          | 0.1 : 8 : 20 |                         | 2.53 | — | — | |
| Epothilone B     | Epothilone B | MAA | EGDMA | AIBN | Methanol:acetonitrile | 1 : 2 : 20 | Bulk polymerisation | 1.28 | — | — | — |
|                   |     |     |     |       |          | 1 : 4 : 20 |                         | 1.98 | — | — | |
|                   |     |     |     |       |          | 1 : 6 : 20 |                         | 1.61 | — | — | |
|                   |     |     |     |       |          | 1 : 8 : 20 |                         | 1.33 | — | — | |
|                   |     |     |     |       |          | 1 : 10 : 20 |                        | 1.24 | — | — | |
| Sulfa-dimethoxine| Sulfa-dimethoxine | MAA | EGDMA | AIBN | Acetonitrile | 1 : 4 : 20 | Bulk polymerisation | 2.63 | — | — | — |
|                   |     |     |     |       |          | 1 : 6 : 20 |                         | 3.94 | — | — | |
|                   |     |     |     |       |          | 1 : 15 : 20 |                        | 1.14 | — | — | |
|                   |     |     |     |       |          | 1 : 15 : 40 |                       | 0.89 | — | — | |
| Hydrochlorothiazide| Hydrochlorothiazide | AAm | EGDMA | AIBN | Tetra-hydrofuran | 0.5 : 1 : 10 | Bulk polymerisation | 1.56 | — | 55.6 | Not mention their specific application in the article, but could be used for any application which needed a sorbent to purify and concentrate target analyte |
|                   |     |     |     |       |          | 0.5 : 2 : 10 |                         | 8.04 | — | 242.6 | |
|                   |     |     |     |       |          | 0.5 : 3 : 10 |                         | 2.80 | — | 189.9 | |
|                   |     |     |     |       |          | 0.5 : 4 : 10 |                         | 1.86 | — | 185.6 | |
| Ethyl adenine-9-acetate (EA9A) | Ethyl adenine-9-acetate (EA9A) | MAA and MMA | EGDMA | AIBN | Acetonitrile | 0.36 : 8.6 : 0.62 | Bulk polymerisation | — | 10.6 (µmol/g) | — | Chromatographic applications |
|                   |     |     |     |       |          | 0.14 : 8.6 : 0.62 |                         | 9 (µmol/g) | 7 (µmol/g) | — | |
|                   |     |     |     |       |          | 0.07 : 8.6 : 0.62 |                         | 6.9 (µmol/g) | 11 (µmol/g) | — | |
|                   |     |     |     |       |          | 0.36 : 8.6 : 0.62 |                         | 11 (µmol/g) | 12 (µmol/g) | — | |
|                   |     |     |     |       |          | 0.36 : 8.6 : 23 : 49 |                       | — | — | — | |
| Nitrofuran (NFT) | 3-Carboxybenzyl-1-l-methineamino-2,4-imidazolidinedione (CPAH) | BMP a | PETRA a | Irgacure 127 | DMSO 1-acetonitrile (67 : 33) | 1 : 1 : 12 | Bulk polymerisation | 2.47 | 5.09% | — | Not mention their specific application in the article, but could be used for any application which needed a sorbent to separate target analyte |
|                   |     |     |     |       |          | 1 : 4 : 20 |                         | 2.49 | 6.20% | — | |

a) T = template; fM = functional monomer; X = crosslinker; IF = imprinting factor; $K_d$ = distribution coefficient; MAA = methacrylic acid; AAm = acrylamide; AAc = acrylic acid; BMP = 2,6-bis(methacrylamido) pyridine; TRIM = trimethylolpropane trimethacrylate; EGDMA = ethylene glycol dimethacrylate; DVB = divinylbenzene; PETRA = pentaerythritol triacrylate; AIBN = azobisisobutyronitrile; DMSO = dimethyl sulfoxide.
Joke Chow and Bhawani aimed to synthesise MIPs in the form of microspheres with high binding capacity for cinnamic acid. They prepared three MIPs, with cinnamic acid as the template, acrylic acid as the monomer, and divinylbenzene as the crosslinker. MIPs polymerisation adopted a non-covalent approach. MIPs polymerisation adopted a non-covalent approach. The Non-covalent approach has a high-affinity binding site to the complex formed between cinnamic acid and acrylic acid. The template:monomer:crosslinker ratios were 1:6:20 (MIP1), 1:4:20 (MIP2), and 1:6:28 (MIP3). MIP1 had the highest IF (3.842). The authors illustrated that as the monomer amount increased, the number of binding sites in polymer also increased. A crosslinker is often used to increase the mechanical stability of polymers. MIP1 could rebind up to 80.7% of cinnamic acid compared to other MIPs and NIPs. The extraction of cinnamic acid from human plasma by MIP1 was effective. The extraction efficiency of MIP1 to cinnamic acid from spiked plasma was over 75%.

Arbazadeh and Abdouss synthesised MIPs for pseudoephedrine separation. The authors used the MIPs for SPE and prepared them with a precipitation polymerisation method. They employed methacrylic acid as the functional monomer and EDGMA as the crosslinker. MAA has carboxyl functional groups and form hydrogen and electrostatic interactions with pseudoephedrine. They prepared five MIPs with different template:monomer:crosslinker ratios: 1:2:63 (MIP1), 1:3:63 (MIP2), 1:4:63 (MIP3), 1:6:63 (MIP4), and 1:8:63 (MIP5). MIP1 had the greatest IF (20). The authors confirmed imprinting-induced extraction by the determination of recovery values for NIP1 (4%) and MIP1 (80%). They found that the concentration of pre-polymerised complex can be amplified by increasing the concentration of template; this augmentation does not greatly influence the polymer structure. Increasing the number of functional monomers resulted in greater non-specific affinity. The impact of non-specific interactions at recognition sites in the MIP of pseudoephedrine in biological fluids was studied with MIP and NIP materials. The MI-SPE binding assay for 10^{-4}M pseudoephedrine in urine was 80% for MIP1 and 1.5% for NIP1 (1.5%); in human serum; the values were 80% for MIP1 and 1.8% for NIP1. These data indicate the high selectively of the generated MIP. This selective analytical method provides a basis for fabricating MIPs for pharmacy and for fast and reliable medical analysis of biological fluids.

Roland and Bhawani synthesised MIP microspheres for piperine, with piperine as the template molecule, acrylic acid as the functional monomer, and EDGMA as the crosslinker. Piperine and acrylic acid interact by non-covalent interactions in the pre-polymerisation mixture. They prepared four MIPs with the following template:monomer:crosslinker ratios: 1:4:16 (MIP1), 1:4:24 (MIP2), 1:6:16 (MIP3), and 1:6:24 (MIP4). MIP3 had the greatest IF (2.86), likely because there was more monomer in it compared with MIP1 and MIP2. The authors explained that as the monomer amount increased, there were more specific interaction sites with piperine as well as increase rebinding efficiency. Although MIP4 had the same monomer ratio as MIP3, its relatively low binding capacity was due to the high crosslinker amount. Yilmaz et al. also stated that higher monomer:crosslinker ratios in MIP preparation will exert proportional changes in recognition capabilities compared with other MIPs prepared at lower monomer:crosslinker ratios. A lower IF is caused by fewer good-quality binding sites. For Roland and Bhawani, MIP3 exhibited the highest binding capacity, up to 84.94%, compared with the other MIPs and NIPs. MIP3 extracted about 81.18% of piperine from spiked urine. These MIPs are used in extraction of piperine and use as sorbent to separate target analyte.

Bakhtiar et al. synthesised MIPs for selective extraction of 2-phenylphenol. The template molecule of these MIPs was 2-phenylphenol, the functional monomer was styrene, and the crosslinker was divinylbenzene. Two-phenylphenol and styrene complex is formed in situ by non-covalent interactions. The non-covalent interactions such as hydrogen bonding and hydrophobic interactions between 2-phenylphenol and styrene will give a good imprint on the polymer. The authors employed precipitation polymerisation to generate the MIPs. The template:monomer:crosslinker ratios were 1:2:16 (MIP1), 1:3:16 (MIP2), and 1:4:16 (MIP3). MIP3 had the greatest IF (2.14), compared with 2.04 for MIP2 and 1.87 for MIP3. MIP3 had a higher amount of functional monomer compared with the other MIPs, and thus there were more specific binding sites and increased binding efficiency. MIP3 extracted 2-phenylphenol with 93% recovery in spiked human blood and 88% recovery in water. These MIPs could be useful for SPE. These MIPs could be useful as SPE sorbent, and analysis in biological and environmental field.

Shafqat et al. synthesised MIPs for Congo red extract-

Table 2. The Interaction Mode between Template and Functional Monomer

| Template molecule                | Functional monomer | Interaction mode          |
|----------------------------------|--------------------|---------------------------|
| Ciproflaxacin                    | MAA                | Non-covalent              |
| Erythromycin                     | MAA                | Hydrogen and ionic bond   |
| Glitenclamide                    | AAm                | Hydrogen bond             |
| Cinnamic acid                    | AAc                | Non-covalent              |
| Pseudoephedrine                  | MAA                | Hydrogen bond and electrostatic interactions |
| Piperine                         | AAc                | Non-covalent              |
| 2-Phenylphenol                   | Styrene            | Non-covalent              |
| Congo red                        | MAA                | Hydrogen bond             |
| Epothilone B                     | MAA                | Hydrogen bond             |
| Sulfadimethoxine                 | MAA                | Hydrogen bond             |
| Hydrochlorothiazide              | AAm                | Hydrogen bond             |
| Ethyl adenine-9 acetate (EA9A)   | MAA and MMA        | Non-covalent              |
| 3-Carboxybenzyl-1-methineamino-2,4-imidazolidinedione (CPAH) | BMP                | Hydrogen bond             |
tion, with MAA as the functional monomer and EDGMA as the crosslinker. MAA establishes a hydrogen bond with the Congo red in the pre-polymerization mixture. The template:monomer:crosslinker ratios were 0.1:4:20 (MIP1), 0.1:6:20 (MIP2), and 1:8:63. MIP1 had the highest IF (2.80) compared with 2.68 for MIP2 and 2.53 for MIP3. Increasing the polymer concentration will decrease the removal efficiency of the MIP and lower the IF due to aggregation of the polymer particles, with a consequent decrease in adsorption. Increasing the polymer concentration diminished the accessible binding sites (fewer available binding sites). This phenomenon decreased the removal efficiency. MIP1 effectively extracted Congo red from dissimilar aqueous media; the extraction efficiency was up to 90% from spiked water samples. These MIPs could be beneficial in analysis in environmental field.69)

Gong et al.70) synthesised and characterised MIPs for epothilone with bulk polymerisation. They used epothilone as the template molecule, MAA as the functional monomer, and EGDMA as the crosslinker. MAA has carboxyl functional groups and the interactions of epothilone B with MAA involve hydrogen bonding. The template:monomer:crosslinker ratios were 1:2:20, 1:4:20, 1:6:20, 1:8:20, and 1:10:20. They observed the highest IF (1.98) at the 1:4:20 ratio and the lowest (1.24) at the 1:10:20 ratio. Tom et al.21) explained that complementarity between the template and cavity in a polymer is reduced by adding too much functional monomer. He et al.71) also stated that producing a highly stable polymer depends on a strong interaction form between the template and functional monomer: the stronger this interaction, the greater the number of specific recognition sites in the MIP. The selectivity of MIPs for epothilone is better based on the study of adsorption properties through static adsorption and also Scatchard analysis was performed. The application of these MIPs could be used as a sorbent to purify and concentrate epothilone.70)

Tom et al.21) synthesised MIPs for sulfadimethoxine separation. They prepared MIPs by using sulfadimethoxine as the template, MAA as a functional monomer, and EGDMA at the crosslinker. Sulfadimethoxine has at least six locations of potential hydrogen bonding that formed with MAA in polymerisation process. This investigation illustrated that the higher functional monomer:template ratio (6:1) produced a greater IF compared with a lower ratio (4:1). This difference was caused by increasing the amount of non-covalent interactions formed between the template and functional monomer. They authors indicated that MIPs prepared with a slight excess of monomer in solution during polymerisation can optimise the number of interactions between the template and functional groups of the functional monomer; this phenomenon produces more binding sites in the MIP. However, a MIP with too much functional monomer may decrease the complementarity between the template and cavity. This eventuality occurs either as a result of a reduction in the number of binding sites in the cavity or altered polymer rigidity and fewer cavities formed by the template. On the other hand, this study also illustrated that the lower crosslinker:monomer ratio (20:15) resulted in a higher IF than a higher ratio (40:15). A slight excess of the crosslinker in the solution also improved the imprinting process because a higher percentage of template and monomer interactions increase the number of binding sites. A higher amount of crosslinker used to generate a MIP might decrease the number of interactions between the template and monomer, with a consequent reduction in the effectiveness of the cavities and reduced number of binding sites in the polymer. On the other hand, a higher amount of the crosslinker will form a very rigid polymer to permit large template molecule to enter the cavity be held efficiently. With further optimisation, these MIPs might be suitable in SPE for purification and concentration of sulfadimethoxine as well as in HPLC-based analysis.21)

Barros et al.72) synthesised MIPs for hydrochlorothiazide. The MIPs were used as a SPE sorbent. The authors prepared the MIPs using hydrochlorothiazide as the template, acrylamide as the functional monomer, and EDGMA as the crosslinker. The complex between hydrochlorothiazide and acrylamide was established by non-covalent interactions such as hydrogen bonds, ionic bonds, van der Waals forces and/or hydrophobic effect forces, but particularly by hydrogen bonds. The thiazide ring and two interaction sites at the sulfonamide functional group can form hydrogen bonds. The highest IF was 8.04, obtained at the template:monomer:crosslinker ratio of 1:4:20. A smaller amount of monomer (i.e., 1:2:20) resulted in a lower IF (1.56). An excess amount of monomer (i.e., 1:8:20) also lowered the imprinting effect (IF = 1.86). Barros et al.72) explained that a large amount of functional monomer typically results in more non-specific interaction sites. Meanwhile, inadequate functional groups produce less complexation in the polymerisation process. The appropriate amount of functional monomer is crucial in the MIP preparation to enhance the specific affinity of the polymers as well as the number of recognition sites. The MIP with a 1:4:20 component ratio recovered up to 95.3% of hydrochlorothiazide. The MIP effectively was used for the SPE of hydrochlorothiazide in a compositive urine sample. These MIPs could use as sorbent to separate target analyte.72)

Athikomrattanakul et al.73) synthesised MIPs for nitrofurantoin detection. In the MIP preparation, the authors used carboxyphenyl aminoxydantoin (CPAH) as the template, 2,6-bis(methacrylamido)pyridine (BPM) as the functional monomer, and pentaerythritol triacrylate (PETRA) as the crosslinker. BPM formed hydrogen bond with CPAH pseudoephedrine. The hydrogen is formed between the imide functional group of CPAH with the protons of the pyridine functional group of BPM. MIP1, with a template:monomer:crosslinker ratio of 1:4:20, had the highest IF (2.49), compared with MIP2 (2.37), which had a 1:1:12 component ratio. The authors explained that MIP2 had the higher binding efficiency because it was prepared with more functional monomer. The main recognition and binding process of a MIP is determined by the interaction between the functional monomer and functional group. The binding capacity of nitrofurantoin was 5.09% for MIP1 and 6.20% for MIP2; these values are insufficient for the sensor arrangement application but could use as sorbent to separate target analyte.73)

The IF is one of the criteria used to evaluate a MIP’s performance. It is a crucial parameter in the application of MIPs in sensors (chemical/biological sensing).22) A high IF value may be achieved by minimising the composition of the monomer. A MIP with excess functional monomer primarily binds by non-specific interactions. The non-specific sites affect the recognition and binding of a MIP to an analyte, with a smaller IF. A high IF indicates that imprinting was successful and the
MIP should selectively bind to analytes in great excess compared to non-selective binding in an NIP.55 The value of an IF can represent the quality of sample separation: The greater the IF, the better the process of separation and recognition between analyte and interferents. A good separation profile of a MIP is certainly required for applications, especially SPE and HPLC analysis.

3.2. Sorbent Binding Capacity A MIP for sensor applications should have a high binding capacity to capture a target compound from the sample.74 In the case of a macromolecule sensor, rebinding capacity is important because macromolecules have a large size, so its desorption efficiency might be low.75 Adsorbents with a high binding capacity are highly desirable in industrial fields. Therefore, a small number of adsorbents is sufficient to perform separations and purification so that solvent, energy, costs, and waste are minimised.76 For this reason, a MIP is hypothesised to greatly enhance the binding capacity and kinetics while providing sufficient recognition sites on the imprinted polymer.77

The optimal binding capacity may be obtained by maximising the composition of the functional monomer and minimising the composition of the crosslinker. Rampey et al.78 showed that a MIP for ethyl adenine-9-acetate (EA9A) with higher concentrations of the template and lower concentrations of crosslinker had greater binding capacities due to the presence of the template, with more high-affinity sites. Increasing the template amount in the polymerisation mixtures increases the proportion of high- to low-affinity sites. EA9A established a non-covalent interaction with MAA through formation of complexes. The MIPs could be useful in chromatographic applications. In contrast, Athikomrattanakul et al.79 showed that a polymer prepared with a higher concentration of functional monomer and crosslinker but a lower concentration of target template still has a relatively high binding efficiency for recognition nitrofurantoin with CPAH as the polymer template. The authors stated that this phenomenon might occur due to the higher concentration of the functional monomer. Meanwhile, Joke Chow and Bhaman96 determined that a MIP for cinnamic acid synthesised with a lower amount of crosslinker reached a higher binding efficiency compared with the one with a higher crosslinker amount at the same template and monomer concentrations. The study also reported that a MIP with a higher monomer ratio achieved the highest binding efficiency compared to a lower monomer ratio with the same amount of template and crosslinker. The authors stated that this outcome might have occurred because MIP with a high amount of monomer and a low amount of crosslinker contains complementary binding sites with cinnamic acid. Increasing the amount of functional monomer will also amplify the possibility of binding sites. On the other hand, a crosslinking agent is commonly used to increase the mechanical stability of polymers.

3.3. Sorbent Selectivity Selectivity is a critical parameter to assess the characteristics of a MIP as a sensing material, or for any other application. The selectivity of imprinting reactions plays a critical role in the selectivity of molecularly imprinted sensors. High matrix complexity requires a more specific and sensitive recognition system. A proper sensor is determined by its excellent specificity and selectivity, recognition, low limit of detection (LOD) and limit of quantitation (LOQ), robustness, and quality of the results achieved. A MIP that fulfills these requirements might improve sensor-based analysis methods.25,26 In MI-SPE, a highly selective sorbent to target molecule will preferentially retain the target compared with other molecules present in the sample. Hence, there will be better quantification of the target analyte.79 The distribution ratio \((K_d)\) is estimated to investigate a MIP’s selectivity.67 A higher \(K_d\) indicates favourable adsorption, and the solute preferentially migrates from the solution to the binding sites of the imprinted polymer.79

Higher sorbent selectivity occurs for MIPs with a template:monomer ratio of 1:4. Barros et al.72 study on hydrochlorothiazide illustrated that the highest \(K_d\) \((242.6 \text{ mL/g})\) occurred at the template:monomer:crosslinker ratio of 1:4:20 with. Less monomer \((i.e., 1:2:20)\) resulted in a lower \(K_d\) \((55.6 \text{ mL/g})\). An excess amount of monomer \((i.e., 1:6:20 and 1:8:20)\) also had a lower \(K_d\) values \((189.9 \text{ and } 185.6 \text{ mL/g})\), respectively. Greater specificity of the recognition sites is a result of favourable and more stable interactions of the target template hydrochlorothiazide with monomer and porogen. The minimum amount of monomer used in the polymerisation process resulted in only a small amount of hydrochlorothiazide that could create stable complexes; hence, most of the hydrochlorothiazide molecules remained unbound in the free state. A MIP prepared with excess monomer primarily rebinds via non-specific interactions. Those non-specific sites affect the selectivity of MIP, reflected by lower \(K_d\) values. The high distribution coefficient presented better quantification of the hydrochlorothiazide compared to the analog compound.72

Higher sorbent selectivity performance is also obtained by using a minimal amount of crosslinker. Liu et al.62 showed that the \(K_d\) of a MIP for CIP was 0.81 \text{ mL/g} at template:monomer ratio of 1:20. The \(K_d\) increased to 41.64 \text{ mL/g} at a 1:16 template:monomer ratio. A highly crosslinked net could inhibit the mass transfer of the imprinted molecule in the polymerisation process and, therefore, prevent the imprinted molecule binding to polymer recognition sites because imprinted molecule is not allowed to enter the interior of the polymer thus reduce selective recognition sites. Optimal sorbent selectivity is achieved by MIPs prepared with high concentrations of monomer and a minimum concentration of crosslinker. However, the excess amount of monomer would not be directly proportional to any improvement in sorbent selectivity. Optimal sorbent selectivity might lead to a convenient adsorption capability and selective recognition characteristics for the target sample in the extraction process of CIP from animal-derived foods.

4. Conclusion and Future Prospects This review has demonstrated that ratios between functional monomer, target template, and crosslinker in the preparation process influence MIP properties, including affinity and imprinting efficiency. A low amount of monomer might decrease the binding sites in the synthesised polymer due to fewer template and monomer complexes formed, while a high amount of monomer might reduce binding selectivity as a result of higher non-specific binding. Crosslinkers also affect MIP selectivity and binding capacity. A MIP that is synthesised with a small amount of crosslinker has a low crosslinking degree that make it cavity configuration unstable. An excessive amount of crosslinker, on the other hand, diminishes the number of recognition sites in a MIP. From all reports in this review,
the synthesis of MIP using a template:monomer:crosslinker ratio of 1:4:20 is more likely to provide optimal imprinting efficiency.

Molecular imprinting technologies currently have been applied in numerous fields in analytical application such as biological field, environmental field, and chemical sensor. However, despite the number of its applications, there is only a limited number of MIP applications in food analysis found in literature. Therefore, it is still necessary to study the effect of MIP ratio in food analysis application. In this regard, further optimisation of MIPs and transducers are encouraged and required. For the imprinting efficiency of these applications, it will be suitable to develop a molecular imprinting method to maximise MIP imprinting efficiency. Further studies to optimise MIP composition to maximise the imprinting effect and optimise selectivity of MIP might result in a polymer that could directly use in separation analysis such as HPLC or SPE.

**Conflict of Interest** The authors declare no conflict of interest.

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