



# A Review on Molecularly Imprinted Polymer (MIP) for Electrochemical Sensor Development

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**Abstract** Molecularly imprinted polymers (MIPs) technology has been studied extensively for multiple applications including analyte detection and chemical separation in the field of medical, pharmaceutical, food safety, and environment. Electrochemical sensors were benefitted from MIPs technology due to their chemical and physical robustness, high sensitivity, selectivity and stability, simple fabrication process, and low-cost of production. The incorporation of MIPs has allowed the development of sensors without biological elements. However, the optimization of the imprinted products requires optimal synergistic effect of multiple factors including materials selection and synthesis techniques. This optimization will form specific recognition cavities for template molecules in the polymeric matrix. This manuscript presents a summary of various MIPs synthesis techniques and performance analysis based on recent studies. The challenges faced in MIPs technology were also discussed to help future researchers in improving technology and boosting commercialization potential against the conventional electrochemical sensor.

**Keywords:** molecularly imprinted polymer, electrochemical sensor, polymerization.

## Introduction

Molecularly imprinted polymer (MIP) is a highly selective polymeric matrix that is obtained from the use of molecularly imprinted technology (MIT). The MIP technique utilizes the designing of an artificial receptor with pre-decided selectivity and specificity for a particular analyte (Vasapollo *et al.*, 2011; Liu *et al.*, 2021). The interest in utilizing MIP as a recognition element in sensor and other fields has increased in recent years since MIP does have special characteristics and potential that comprise of high selectivity, high stability, and durability when employed in harsh environment as compared to a biological system (Vasapollo *et al.*, 2011; Zaidi, 2016; Crapnell *et al.*, 2020). Some other potential applications of MIP are in the areas of theranostics, pharmaceutical research, drug delivery (Alvarez-Lorenzo and Concheiro, 2004; Zaidi, 2016; Parisi *et al.*, 2022), separation sciences, and purification (Vasapollo *et al.*, 2011; Li *et al.*, 2019).

The working principle of this polymeric biomimetic recognition element is similar to the concept used in enzymatic reaction and antibody-antigen interaction. This principle employs the concept of the “lock and

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key” during the working process (Zaidi, 2016; Liu *et al.*, 2021). The adaptation is made here by having the MIP as the lock or the biomimetic recognition element and the target molecule (template) as the key in the interaction. The unique feature of the MIP allows it to be more advantageous compared to its biological sibling, whereby it has the ability to be produced at a lower cost and having a longer life-span with good storage stability (Cheong, Yang and Ali, 2013; Li *et al.*, 2019; Md Shakhiih *et al.*, 2021). The high specificity and selectivity (Bt Ahamad Mashat *et al.*, 2022) of MIP towards the target molecule make it a good candidate to replace biological receptors in biosensors and opened its potential in replacing and improving biological system-based biosensors (Farooq *et al.*, 2022; Ramanavičius *et al.*, 2022). Besides its usage in biomolecules detection, MIP can also be used in detecting environmental pollutants and used for water treatment (Sarpong *et al.*, 2019)

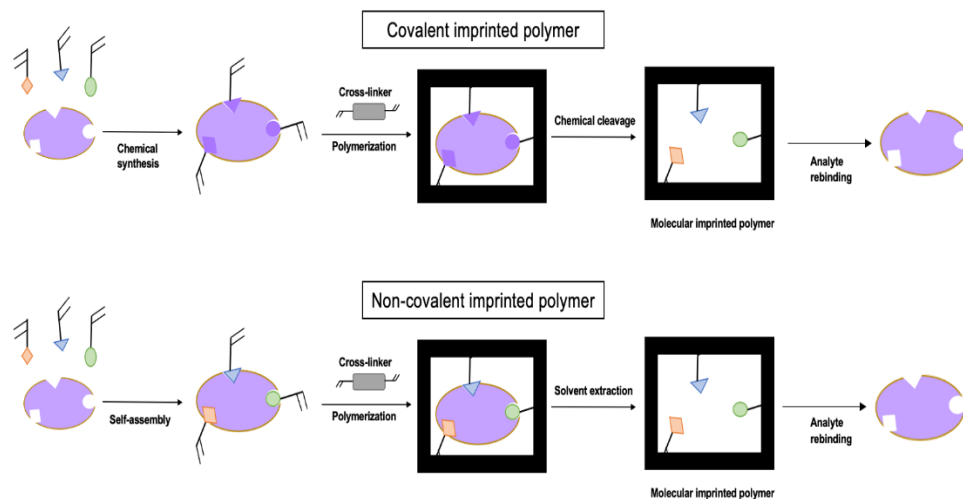
Based on a recent research, scholars have been classifying the receptor and transducer as the basic components of biosensor (Cui, Zhou and Zhou, 2020). The receptor acts as the recognition element by recognizing and selecting the target molecule specifically and the transducer will convert the binding energy into measurable electrical energy (Cui, Zhou and Zhou, 2020). In electrochemical applications, an electrode is normally used as the transducer for the sensor. The general methods that can be performed to produce electrochemical biosensor include the assembly of the functional monomer and the template (target molecule), polymerization of the monomer-template complex with cross-linkers, and template removal forming cavities (Li *et al.*, 2019; Cui, Zhou and Zhou, 2020). In this paper, apart from the applications of MIP, the extensive view of performance analysis for the current implementation of MIP in biosensors is also reviewed by looking into various aspects such as sensitivity, linear range, and limit of detection (LOD) of the sensor. The challenges and limitations faced in MIP technology are also highlighted to provide insights on the future application and improvement in electrochemical sensor development.

### **Synthesis of Molecularly Imprinted**

The general protocol for MIP preparation is usually done with a reaction mixture composing of a template, a functional monomer, a cross-linker, and a polymerization initiator dissolved in an appropriate solvent (Cheong, Yang and Ali, 2013; Zaidi, 2016; Li *et al.*, 2019). The selection and composition of these elements and polymerization techniques used in the preparation of the MIP will influence the characteristics and performance. This will result to MIP giving the freedom and flexibility in pre-determining the desired properties depending on its application (Liu *et al.*, 2021). The interaction between the functional group of the monomers and the template molecule will create a pre-polymerization complex comprising of the polymerizing units and template recognition element (Adumitrăchioaie *et al.*, 2018; Tio *et al.*, 2018). The formation of the pre-polymerization complex between the monomers and template molecules (Parisi *et al.*, 2022) has three different nature of bonding which are covalent, semi-covalent, and non-covalent bonding (Sarpong *et al.*, 2019; Farooq *et al.*, 2022). A sufficient interaction between monomer and target molecule is essential in producing MIP with networks of working recognition sites.

Covalent (pre-organized approach) reversible bonding between the monomer and target molecule will give off the population of binding sites that are rather homogenous, help with lowering binding sites that are non-specific, and producing stable binding sites (Vasapollo *et al.*, 2011; Sarpong *et al.*, 2019; Parisi *et al.*, 2022). However, this approach is not the most popular approach because the removal of the template molecule requires complicated steps in order to cleave the covalent bonds due to the strong chemical bonding interaction and has slower binding (Vasapollo *et al.*, 2011; Zaidi, 2016; Sarpong *et al.*, 2019; Fresco-Cala, Batista and Cárdenas, 2020). In 2002, Hwang & Lee prepared cholesterol imprinted polymer using two different monomers with two methods of imprinting being covalent and non-covalent (Hwang and Lee, 2002). The MIP by covalent imprinting uses cholesteryl (4-vinyl) phenyl carbonate as its monomer while the MIP by non-covalent imprinting method uses methacrylic acid as the monomer (Hwang and Lee, 2002). Both methods show good performance but, the covalently imprinted MIP in comparison to non-covalent shows higher adsorption capacity for cholesterol and about fivefold higher chromatographic efficiency for cholesterol separation (Hwang and Lee, 2002). The covalent imprinting method also notably lowered the peak broadening and tailing (Hwang and Lee, 2002).

Alternatively, is the non-covalent (self-assembling approach) bonding that uses the slightly weaker interactions such as hydrogen bonds, Van der Waals forces, ion or hydrophobic interaction, and metal-coordination (Vasapollo *et al.*, 2011; Sarpong *et al.*, 2019; Fresco-Cala, Batista and Cárdenas, 2020). This approach is less complex, cheaper, can produce high recognition sites, and has the flexibility of functional monomer being able to interact with almost any type of template molecule (Vasapollo *et al.*, 2011; Sarpong *et al.*, 2019). Nevertheless, this approach also has some limitations that include low-selectivity of binding sites and low binding affinity at the binding sites (Vasapollo *et al.*, 2011; Sarpong *et al.*, 2019). Figure 1 shows a comparison of the mechanism between covalent and non-covalent molecular imprinting. In recent studies, researchers have implemented the non-covalent bonding between the functional monomer being methacrylic acid (MAA) and template molecule being atrazine (Ahmad, Lah and Low, 2018). In this complex binding site formation, dual hydrogen bonds were formed between atrazine and MAA (Ahmad, Lah and Low, 2018). The carboxylic group (-COOH) of the MAA acts as both hydrogen bond receptor and donor which interacts with the hydrogen atom of an amino group from atrazine and the nitrogen atom of the atrazine (Ahmad, Lah and Low, 2018). Based on the study, it can be observed that the molarity of the monomer can affect the binding affinity of the MIP. Higher molarity of the monomer can cause the imprinting effect to be rather poor (Ahmad, Lah and Low, 2018). Yet, the sensitivity of the system is still good since it can detect a low level of atrazine concentration (Ahmad, Lah and Low, 2018).



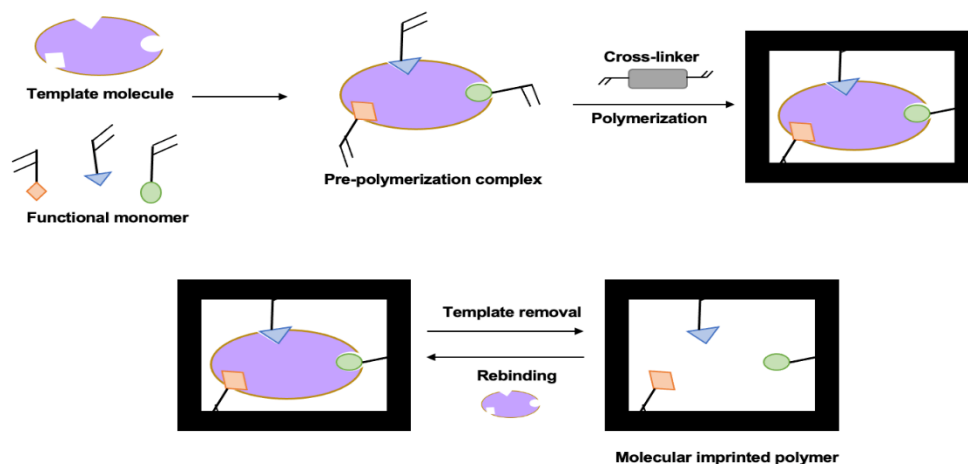
**Figure 1.** Representation of mechanism for covalent and non-covalent molecular imprinting.

Another method of pre-polymerization technique is the semi-covalent approach that uses the combination of both covalent and non-covalent bonds. In this approach, covalent bonding is used in the formation of a pre-polymerization complex between monomer and template molecule while non-covalent bonding is used in the stage of rebinding the template molecule (Adumitrăchioaie *et al.*, 2018; Sarpong *et al.*, 2019; Fresco-Cala, Batista and Cárdenas, 2020). Thus, it has the properties of both covalent and non-covalent bonds. The binding sites produced are rather uniformly distributed but there is also a possibility of template bleeding if hydrolysis could not remove the template (Sarpong *et al.*, 2019). In 2015, a study was conducted to prepare thiophene-based MIP for human serum albumin (HSA) determination using semi-covalent imprinting method (Cieplak *et al.*, 2015). Two different functional monomers which are the carboxylic acid part and the amine group were covalently bound to the respective functional groups on the surface of the HSA (Cieplak *et al.*, 2015). In the rebinding process, the HSA molecules bound to the active sites of MIP only by non-covalent bonding (Cieplak *et al.*, 2015). According to them, the results show high imprinting factors reached which strengthens the hypothesis that the semi-covalent imprinting method is successful here. This results accepted the hypothesis with

the formation of a large number of very well-defined molecular cavities with high affinity to the HSA molecules (Cieplak *et al.*, 2015). The MIP also shows high selectivity against interferences such as blood and urea (Cieplak *et al.*, 2015).

### Polymerization Techniques

Besides the optimal composition of the pre-polymerization process, the selection of polymerization technique during the preparation of MIP sensor is critical to create sensors with the desired performances and properties. Generally, polymerization process follows the formation of a pre-polymerization complex between the template and the functional monomer. An organic cross-linker (rarely inorganic) is then added to the complex mixture to ensure the binding of the monomers surrounding the template molecules which then will results in a three-dimensional polymer matrix with the template molecules trapped in it (Cheong, Yang and Ali, 2013; Adumitrăchioaie *et al.*, 2018). Polymerization is a chain of reaction that requires an activator for it to happen. Initiator which is an activation element is added to the complex mixture to help triggering the polymerization process (Adumitrăchioaie *et al.*, 2018; Tio *et al.*, 2018). Finally, extraction of the template molecules is done by thoroughly washing the matrix using mild acid, leaving permanent cavities inside the matrix complementary to the size, shape, and molecular interaction of the template (Cheong, Yang and Ali, 2013; Zaidi, 2016; Denmark, Mohapatra and Mohapatra, 2020). The cavities having the complementary shape, size, and surface chemistry to the template will allow it to have high affinity and selectivity towards the target template molecule (Denmark, Mohapatra and Mohapatra, 2020). Generally, MIP polymerization can be done either by free radical polymerization or sol-gel process. However, with free radical polymerization has been the more common method amongst the two. A few types of free radical polymerization techniques are available in preparing electrochemical-based MIP sensors such as traditional (bulk) polymerization, precipitation polymerization, suspension polymerization, and in situ polymerization. The fundamental steps for MIP polymerization process is shown in Figure 2.



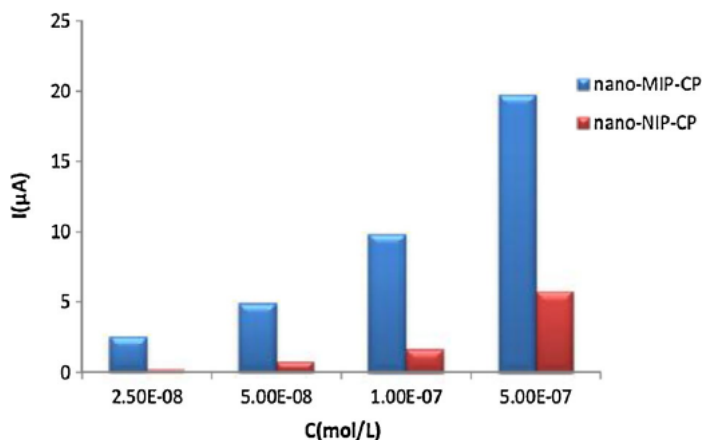
**Figure 2.** Fundamental steps of polymerising molecular imprinted polymer

Traditional (bulk) polymerization is one of the most frequently used methods in preparing MIP sensors. This method is done by synthesizing the MIP in bulk and followed by mechanically grinding and sieving the particles into obtaining the desired size range according to its application (Vasapollo *et al.*, 2011; Chen *et al.*, 2016a; Sarpong *et al.*, 2019). The benefit of this method is that it is simple to prepare, low cost, and the size of the particles can be controlled. However, this method also has plenty of drawbacks such as low binding capacity resulted from some of the binding sites being destroyed in the preparation process, the irregular size and shape of the particles, requiring a high volume of the template molecules, as well as the grinding and crushing of the particles itself is tedious and time-consuming (Vasapollo *et al.*, 2011; Chen *et al.*, 2016a; Sarpong *et al.*, 2019). In a previous study, the authors created a simple and disposable sensor for the electrochemical detection of paraben using amino acid-based monomer on screen-printed gold electrode surface in the presence of polyvinyl alcohol (PVA) (Yücebaş *et al.*,

2020). The use of bulk polymerization technique on surface imprinting is one of the strategies that is widely practiced to overcome some of its limitations (Sarpong *et al.*, 2019). The MIP layer was prepared by bulk polymerization performed using a UV lamp (100 W, 365 nm) for 30 min (Yücebaşı *et al.*, 2020). The resulted sensor performed well with a low detection limit at 0.706  $\mu\text{M}$ , while the linear working range was around 1–30  $\mu\text{M}$  at optimum condition (Yücebaşı *et al.*, 2020).

Another type of free radical polymerization is precipitation polymerization. Precipitation polymerization is also known as heterogeneous solution polymerization is quite similar to bulk polymerization but it is widely used to overcome some drawbacks of bulk polymerization (Sun *et al.*, 2018; Sarpong *et al.*, 2019). In this method, the monomers, cross-linkers, and initiators used are dissolved in dispersant to develop into a homogenous mixed solution (Sun *et al.*, 2018; Parisi *et al.*, 2022). The polymer chains will continue to develop and grow into precipitation where they will be too large to be soluble in the reaction solution, then the polymer beads can be easily retrieved and washed (Vasapollo *et al.*, 2011; Sun *et al.*, 2018; Sarpong *et al.*, 2019). As compared to bulk polymerization, this technique is easier, less time-consuming, can produce uniform and regular MIP beads as well as not requiring any surfactants or stabilizers (Vasapollo *et al.*, 2011; Adumitrăchioaie *et al.*, 2018; Sun *et al.*, 2018). Based on a research in 2017, MIP is synthesized using precipitation polymerization using 2,4-DCP, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), and azobisisobutyronitrile as template molecules, functional monomers, initiators, and crosslinkers respectively (Liang *et al.*, 2017). Hence, to detect 2,4-DCP, the glassy carbon electrode was modified by 2,4-DCP MIP and graphene oxide (Liang *et al.*, 2017). It produces results with a good linear range of  $4 \times 10^{-9}$ – $1 \times 10^{-5}$  M and a detection limit of  $5 \times 10^{-10}$  M (Liang *et al.*, 2017). In the same year, another scholars fabricated  $\text{Bi}^{3+}$  ion-imprinted polymer using the same precipitation polymerization method (Alizadeh *et al.*, 2017). For the selective recognition of  $\text{Bi}^{3+}$ , the prepared polymer was used as a modifier to adjust a carbon/carbon nanotube paste electrode to prepare an electrochemical sensor (Alizadeh *et al.*, 2017). It exhibits excellent linearity in the range of  $2 \times 10^{-7}$ – $2 \times 10^{-6}$  M (Alizadeh *et al.*, 2017).

Suspension polymerization is a particularly simple procedure of producing porous MIP (Chen *et al.*, 2016a; Sarpong *et al.*, 2019). In this process, the monomers involved are either insoluble or partially soluble in water to ensure the insolubility of the monomers after polymerization (Sarpong *et al.*, 2019). The technique also requires monomer dispersion to ensure that polymerization is carried out within monomer droplets (Sarpong *et al.*, 2019). Suspension polymerization ensures that polymer beads are formed uniformly and the greatest advantage of this method is the unrestricted and excellent heat transfer which is ideal for industrial scale-up (Adumitrăchioaie *et al.*, 2018; Sarpong *et al.*, 2019). In contrast, the process tends to generate a large size range of particles from micrometres to millimetres, have lower binding sites due to the disruption of the dispersing medium, and is not appropriate for solid-phase extraction (SPE) applications (Chen *et al.*, 2016a; Sarpong *et al.*, 2019). In a study, an electrochemical sensor for the selective and sensitive determination of diazinon (DZN) pesticides was developed based on molecularly imprinted polymer (MIP) nanoparticles (Motaharian *et al.*, 2016). Diazinon imprinted polymer nanoparticles were synthesized by suspension polymerization and then used for carbon paste electrode (CPE) composition modification to prepare the sensor (Motaharian *et al.*, 2016). The results obtained showed that the carbon paste electrode modified by MIP nanoparticles (nano-MIP-CP) has much higher diazinon adsorption capability than the non-printed polymer nanoparticles based on CPE (nano-NIP-CP) (Motaharian *et al.*, 2016). The comparison between nano-NIP-CP and nano-MIP-CP under optimal condition shows that nano-NIP-CP has lower response compared to nano-MIP-CP as depicted in Figure 3. The proposed sensor showed excellent sensitivity ( $95.08 \mu\text{A L } \mu\text{mol}^{-1}$ ) for diazinon under optimized extraction and analysis conditions with two linear ranges of 0.0025–0.10  $\mu\text{mol L}^{-1}$  and 0.10–2.00  $\mu\text{mol L}^{-1}$  and also a detection limit of 70.00079  $\mu\text{mol L}^{-1}$  (Motaharian *et al.*, 2016).



**Figure 3.** Comparison of the SWV responses obtained at the nano-MIP-CP and nano-NIP-CP electrodes over the studied concentration range of DZN under the optimum conditions (Motaharian *et al.*, 2016)

In situ polymerization technique is used to produce monolithic MIP and it developed using in situ free-radical polymerization 'molding' method. This method takes place directly on the surface of the transducer or immobilized after its production, eliminating the need for time-consuming grinding, sieving, and column packing (Puiu, Jaffrezic-Renault and Bala, 2017; Adumitrăchioaie *et al.*, 2018). This allows easy manipulation of the thickness and density of the MIP layer while producing homogenous and controlled MIP coating (Adumitrăchioaie *et al.*, 2018). After the in situ polymerization, monolithic MIP is expected to improve separation and allow direct analysis with high speed and efficiency (Puiu, Jaffrezic-Renault and Bala, 2017; Adumitrăchioaie *et al.*, 2018). This method is also cost-efficient due to the low amount of template it needs (Puiu, Jaffrezic-Renault and Bala, 2017). Besides, it gives high porosity which led to good permeability, and its high surface area is making it great for both small molecules and large biopolymers (Puiu, Jaffrezic-Renault and Bala, 2017). However, this method requires harsh conditions for the template removal, and the removal and rebinding process is rather slow (Adumitrăchioaie *et al.*, 2018). A study in 2017 introduced a method for dodecyl gallate detection using an electrochemical sensor based on a MIP film on the surface of a glassy carbon electrode (GCE) (Pedroso *et al.*, 2017). The MIP was synthesized in situ using ortho-phenylenediamine as the monomer and electropolymerization process (Pedroso *et al.*, 2017). By using square wave voltammetry, the analytical output of the MIP sensor showed a linear range of  $0.50$  to  $8.0 \times 10^{-9}$  mol L<sup>-1</sup>, with a correlation coefficient of 0.9921 (Pedroso *et al.*, 2017). The detection and quantification limits of the sensor were  $0.22 \times 10^{-9}$  and  $0.67 \times 10^{-9}$  mol L<sup>-1</sup>, respectively (Pedroso *et al.*, 2017). While in 2019, an electrochemical sensor is developed by integrating molecularly imprinted polymer, noble metal nanoparticles, and carbon materials using in situ polymerization technique (Yue *et al.*, 2019). This is for selective and sensitive methods of detecting tertiary butylhydroquinone (TBHQ) for food safety and quality (Yue *et al.*, 2019). The sensor performed admirably when it came to detecting TBHQ in a responsive manner (Yue *et al.*, 2019). The linear range was 0.5–60 g mL<sup>-1</sup>, with a 0.046 g mL<sup>-1</sup> detection limit (Yue *et al.*, 2019). Table 1 summarized the advantages and disadvantages of different polymerization techniques used in developing molecular imprinted polymer sensors.

Recently, MIP also has attracted a great deal of interest and promising high performance in stability, selectivity, and sensitivity for targeted molecules (Yang *et al.*, 2019; Yücebaş *et al.*, 2020; Fu *et al.*, 2021; Md Shakhiah *et al.*, 2021). With the help of MIP, various issues and problems from various areas such as food analysis, drug delivery, cancer detection, pharmaceutical, and clinical analysis can be solved (Arvand, Zamani and Sayyar Ardaki, 2017; Yücebaş *et al.*, 2020).



**Table 1.** Comparison of polymerization techniques.

Polymerization techniques	Advantages	Disadvantages	Reference
<b>Traditional (bulk) polymerization</b>	<ul style="list-style-type: none"> <li>▪ Simple to prepare</li> <li>▪ Low cost</li> <li>▪ Size of the particles can be controlled</li> </ul>	<ul style="list-style-type: none"> <li>▪ Low binding capacity</li> <li>▪ Some of the binding sites being destroyed in the preparation process.</li> <li>▪ Irregular size and shape of the particles</li> <li>▪ Requires high volume of the template molecules</li> <li>▪ Time consuming</li> </ul>	(Vasapollo <i>et al.</i> , 2011; Chen <i>et al.</i> , 2016a; Sarpong <i>et al.</i> , 2019)
<b>Precipitation polymerization</b>	<ul style="list-style-type: none"> <li>▪ Less time consuming and easier procedure</li> <li>▪ Produce uniform and regular MIP beads</li> <li>▪ Not requiring any surfactants or stabilizers</li> </ul>	<ul style="list-style-type: none"> <li>▪ Only when the polymeric chains are long enough to be insoluble in the reaction mixture does precipitation occur.</li> </ul>	(Vasapollo <i>et al.</i> , 2011; Adumitrăchioaie <i>et al.</i> , 2018; Sun <i>et al.</i> , 2018)
<b>Suspension polymerization</b>	<ul style="list-style-type: none"> <li>▪ Polymer beads are formed uniformly</li> <li>▪ Unrestricted and excellent heat transfer</li> <li>▪ Ideal for industrial scale-up</li> </ul>	<ul style="list-style-type: none"> <li>▪ The method produces particles in a wide variety of sizes, from micrometres to millimetres.</li> <li>▪ Low binding sites due to the disruption of the dispersing medium</li> <li>▪ Not appropriate for solid phase extraction (SPE) applications</li> </ul>	(Chen <i>et al.</i> , 2016b; Adumitrăchioaie <i>et al.</i> , 2018; Sarpong <i>et al.</i> , 2019)
<b>In situ polymerization</b>	<ul style="list-style-type: none"> <li>▪ Easy manipulation on the thickness and density of the MIP layer</li> <li>▪ Produce homogenous and controlled MIP coating</li> <li>▪ Cost efficient due to the low amount of template it needs</li> <li>▪ The imprinted polymer is formed on the transducer's surface</li> </ul>	<ul style="list-style-type: none"> <li>▪ Requires harsh conditions for the template removal</li> <li>▪ Removal and rebinding process is slow</li> </ul>	(Puiu, Jaffrezic-Renault and Bala, 2017; Adumitrăchioaie <i>et al.</i> , 2018)

Catechin and rutin is a class of flavonoids (plant pigment) which can be harvested in almost all kinds of vegetables and fruits (El Jaouhari *et al.*, 2020). Specifically, catechin is a natural antioxidant and also can be found in herbs, algae, beverages, and confectionery items, but their contents vary considerably among various sources. (Shukla *et al.*, 2018). Rutin is among the most popular (El Jaouhari *et al.*, 2020) flavonoids for its potential activities and can be abundantly found in passion flower, buckwheat, tea, and apple (Ganeshpurkar and Saluja, 2017). This compound attributes significant attention including anti-cancer, anti-inflammatory, anti-bacterial, and blood vessel protecting (Gullón *et al.*, 2017; El Jaouhari *et al.*, 2020; Fu *et al.*, 2021). The study in 2021 successfully developed an electrochemical sensor using Glassy Carbon Electrode (GCE), reduced Graphene Oxide (rGO), Zeolitic Imidazolate Frameworks-8 (ZIF-8), and green tea leaf, in which this sensor can be used as a sample for catechin detection (Fu *et*

*al.*, 2021). By using an electrochemical analysis, differential pulse voltammetry (DPV), the performance of the fabricated sensor exhibited good selectivity, good linearity, and a low detection limit for catechin (Fu *et al.*, 2021). The mMIPs/rGO-ZIF-8/GCE sensor showed a linear range of 0.01 nmol/L–10  $\mu$ mol/L and LOD of 0.003 nmol/L. Meanwhile, another scholar also developed a highly sensitive MIP sensor using the same electrode modification and analytical method to detect catechin in rutin recognition. This sensor had a good linear relationship in the of range, 0.05–100  $\mu$ M and 0.0005–0.05  $\mu$ M with LOD, 0.0001  $\mu$ M in real solutions (El Jaouhari *et al.*, 2020). Apart from the analysis of linear range and LOD, the reproducibility, acceptable stability and high selectivity exhibited a good performance as well on GCE/ZIF-8/rGO/MIP (El Jaouhari *et al.*, 2020). Hence, both flavonoids perform well in the development of MIP sensor for food analysis.

Prostate-specific-antigen (PSA) is a group of proteins produced by cells of the prostate gland which then become the best serum marker for the detection of prostate cancer (Healy *et al.*, 2007). The sample taken is sent to a laboratory for PSA test measurement. The concentration level of PSA in blood will helps in the assessment and early treatment for prostate cancer (Sikaris, 2011). In 2020, few scholars expanded their research in the development of a reliable bioanalytical method for the sensitive monitoring of prostate-specific antigen (PSA) (Abbasy *et al.*, 2020). In their work, electrically conducting poly [Toluidine Blue (PTB)] was electropolymerized in a pre-formed glutaraldehyde-cysteamine (GA-Cys A) in which PTB become an artificial receptor of the test on the surface of gold electrode (Abbasy *et al.*, 2020). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) also performed in assessing the performance of the MIP bioreceptor for measuring PSA in human plasma (Abbasy *et al.*, 2020). The results revealed an excellent linear response in the range of 1–60  $\mu$ g/L with high affinity, selectivity, and acceptable stability (Abbasy *et al.*, 2020). Additionally, CA-125 is another important serum biomarker used in cancer detection and found in ovarian cells (Rebelo *et al.*, 2019). The level of CA-125 in the bloodstream will be measured during the diagnosis monitoring, early stage of detection, during and after treatment. They constructed an electrochemical sensor by electropolymerization on a gold screen-printed electrode (Au-SPE) and on the SPR gold sensor with monomer pyrrole (Py) (Rebelo *et al.*, 2019). Py was used due to its highly selective material for protein and leads to the great performance of the sensor (Rebelo *et al.*, 2019). A comparison between Square Wave Voltammetry (SWV) as an electrochemical method and surface plasmon resonance (SPR) as an optical method was presented it is concluded that SWV has a better performance of the sensor. Consequently, it showed a low detection limit, 0.01 U/mL, and a larger linear range, 0.01 and 500 U/mL (Rebelo *et al.*, 2019). Therefore, MIP has been proposing the potential biosensor in clinical and biomedical analysis in cancer detection.

Lactate is an antioxidant and a key metabolite formed from the anaerobic metabolism of glucose in the human body (Mengarda *et al.*, 2019; Pereira and Stradiotto, 2019; Md Shakhiah *et al.*, 2021). The assessment of this biomarker is widely applied to various applications such as sports medicine, clinical analysis, and the food industry (Rassaei *et al.*, 2014; Pereira and Stradiotto, 2019; Md Shakhiah *et al.*, 2021). Notably, there was a research on electropolymerization of the o-phenylenediamine (o-PD) on the modified surface of the GCE with reduced graphene oxide (rGO) and gold nanoparticles (AuNP) in the presence of lactic acid (Pereira and Stradiotto, 2019). According to these scholars, the linear response and limit of detection of the sensor were 0.1–15.0 nM and 0.09 nM respectively (Pereira and Stradiotto, 2019). Besides, MIP/AuNP/RGO/GCE exhibited excellent selectivity and the sensitivity of the electrochemical sensing (Pereira and Stradiotto, 2019). Similarly, another scholar demonstrated in their research a potentiometric sensor based on PPy film (PPY: LAC) for the determination of lactate level in different biological fluids (Mengarda *et al.*, 2019). By optimizing the sensor, the wide linear range was obtained in the range of 0.1 & 10.0 mmol L<sup>-1</sup>, a limit of detection (LOD), 81  $\mu$ mol L<sup>-1</sup>, and good selectivity to lactate ions (Mengarda *et al.*, 2019). This study showed the great roles of polypyrrole incorporating in a sample and associated with the potentiometric method indicated a good sign in selective performance (Mengarda *et al.*, 2019). Hence, these developed sensors proposed the potential alternative sensing device for lactate detection by assessing the physical performance. The summarized recent electrochemical MIP sensors is tabulated in Table 2.



**Table 2.** Summary of recent electrochemical MIP sensors.

Electrode Modification	Technique	Sensitivity ( $\mu\text{A } \mu\text{mol}^{-1}\text{L}$ )	Linear Range ( $\text{mol L}^{-1}$ )	LOD ( $\text{mol L}^{-1}$ )	Analyte	Reference
mMIPs/rGO-ZIF-8/GCE	DPV	-	$0.01 \times 10^{-9} - 10 \times 10^{-6}$	$0.003 \times 10^{-9}$	Catechin	(Fu <i>et al.</i> , 2021)
GCE/ZIF-8/rGO/MIP	DPV	-	$0.05 - 100 \times 10^{-6}$ & $0.0005 - 0.05 \times 10^{-6}$	$0.0001 \times 10^{-6}$	Rutin	(El Jaouhari <i>et al.</i> , 2020)
MIP-PEDOT/CFP	DPV	28,458	$0.21 - 300 \times 10^{-9}$	$0.07 \times 10^{-9}$	2,4-DCP	(Maria C G <i>et al.</i> , 2020)
MIP/pTBs/Au	DPV	-	$29.41 \times 10^{-12} - 1.176 \times 10^{-9}$	-	PSA	(Abbasy <i>et al.</i> , 2020)
PHEMA-MAPA) nanofilm	CV	-	$1-30 \times 10^{-6}$	$0.706 \times 10^{-6}$	Paraben	(Yücebaş <i>et al.</i> , 2020)
	SWV					
CPE (MIP-CP) 2	CV	-	$16 \times 10^{-9} - 2.5 \times 10^{-6}$	$5.04 \times 10^{-9}$	QTP	(Motaharian <i>et al.</i> , 2019)
	SWV					
MIP/AuNP/RGO/GCE	DPV	-	$0.1-15.0 \times 10^{-9}$	$0.09 \times 10^{-9}$	Lactic acid	(Pereira and Stradiotto, 2019)
PPy: LAC	Potentiometry	-	$0.1 - 10.0 \times 10^{-3}$	$81 \times 10^{-6}$	Lactate	(Mengarda <i>et al.</i> , 2019)
	CV					
MIP@AuSPE	CV	-	$8.3 \times 10^{-29}$ and $4.15 \times 10^{-24}$	$8.3 \times 10^{-29}$	CA-125	(Rebello <i>et al.</i> , 2019)
	SWV					
MIP/PdAuNPs/ERGO/GCE	CV	-	$3 \times 10^{-6} - 0.36 \times 10^{-3}$	$0.28 \times 10^{-6}$	TBHQ	(Yue <i>et al.</i> , 2019)
	DPV					
MIP/f-MWCNTs/GCE	DPV	1.78	$0.05-100 \times 10^{-6}$	$0.005 \times 10^{-6}$	SY	(Arvand, Zamani and Sayyar Ardaki, 2017)
MIP/f-MWCNT/GCE	CV	-	$0.50$ to $8.0 \times 10^{-9}$	$0.22 \times 10^{-9}$	Dodecyl gallate	(Pedroso <i>et al.</i> , 2017)
	SWV					
MIP/GO/GCE	DPV	-	$0.004 - 10.0 \times 10^{-6}$	$0.5 \times 10^{-9}$	2,4-DCP	(Liang <i>et al.</i> , 2017)
IIP-CP	ASV	112.25	$.2 - 2 \times 10^{-6}$	$8.9 \times 10^{-9}$	Bi <sup>3+</sup>	(Alizadeh <i>et al.</i> , 2017)
Nano-MIP-CP	CV	95.08	$0.0025 - 0.10 \times 10^{-6}$ & $0.10 - 2.00 \times 10^{-6}$	$0.00079 \times 10^{-6}$	DZN	(Motaharian <i>et al.</i> , 2016)
	SWV				Pesticides	

Limit of Detection (LOD), Glassy Carbon Electrode (GCE), 2,4-dichlorophenol (2,4-DCP), reduced Graphene Oxide (rGO), Zeolitic Imidazolate Frameworks-8 (ZIF-8), Differential Pulse Voltammetry (DPV), Cyclic Voltammetry (CV), Carbon Paste Electrode (CPE), Square Wave Voltammetry (SWV), Gold Nanoparticle (AuNP), Polypyrrole (PPy), Electrochemical Impedance Spectroscopy (EIS), Poly (3,4-ethylenedioxythiophene) (PEDOT), Prostate-specific antigen (PSA), Carbon Fiber Paper (CFP), poly Toluidine Blue (pTB), methacrylate-N-methacryloyl-L-phenylalanine) (PHEMA-MAPA), Quetiapine (QTP), Gold Screen-printed Electrode (Au-SPE), Carbohydrate Antigen 125 (CA-125), Tertiary Butylhydroquinone (TBHQ), Electrochemically Reduced Graphene Oxide (ERGO), Sunset Yellow (SY), Multi-walled Carbon Nanotubes (MWCNTs), Graphene oxide (GO), Anodic Stripping Voltammetry (ASV), Bismuth Bi<sup>3+</sup>, Ion Imprinted Polymers (IIP), Carbon paste (CP), Diazinon (DZN)

### ***Challenges in Molecularly Imprinted Polymers***

Despite remarkable achievements attained by the MIPs, there are several challenges that need to be addressed for better development and functionality of MIP. One of the issues is in terms of the reusability of MIPs sensor. MIP should be able to be reused by detaching the solvent (template) using chemicals to regenerate their function for the next use. The sensor should maintain their chemicals and mechanical stability in those template removal solutions. However, most studies still exhibit a limited reuse capability (Cui *et al.*, 2020).

Another issue reported to arise from the use of MIPs is the possibility of non-specific interaction which contributes to the heterogeneity of binding sites (Alenazi, Manthorpe and Lai, 2016; Refaat *et al.*, 2019). This occurs since most MIPs are synthesized in organic media (Bui and Haupt, 2010). The non-specific hydrophobic interaction that occurs need to be suppressed without compromising the specific interaction (Haupt, Dzgoev and Mosbach, 1998). The use of organic additives and/or detergents in the buffer has been proposed to reduce this non-specific reaction while maintaining the structure of the target binding unaltered (Andersson, 2000). A study in 2016 suggested the use of methanol to ameliorated this non-specific competitor interferents (Alenazi, Manthorpe and Lai, 2016). Besides, this could also be improved by strengthening the specific interaction between MIP and template using specific monomers that are capable of stoichiometric interaction with the template via strong interaction (Bui and Haupt, 2010).

Template leakage is another limitation associated with the use of MIPs due to the leaching of templates from the polymer (Fizir *et al.*, 2020). After the polymerization process complete, the template is removed to free the imprinting sites. Consequently, false-positive signal may potentially occur due to a leakage. This leakage can happen when the template extraction is not complete which then will cause the entrapped templates to gradually release from MIPs and interact with target sensing hence causing false-positive signals in sensing (Lenain *et al.*, 2015; Wackerlig and Schirhagl, 2016). One of the approaches to solve this problem is by using dummy templates to ensure that even when there is a leakage, it will not affect the accuracy of MIPs (Chen, Xu and Li, 2011). It is also suggested to use a dummy template since some template molecules are expensive and prone to unwanted compound degradation during polymerization (Kugimiya and Takei, 2008).

Templates represent the analyte of interest in the production of MIPs. Many compounds such as amino acids, steroids, nicotine, drug molecules, and others have successfully been used as templates (Cui *et al.*, 2020). This broad range of molecules shared one common attribute which is a relatively low molecular weight (Mahony *et al.*, 2005). This is because most larger molecules are less rigid and difficult to form well-defined binding cavities during the imprinting process. In addition, larger molecules such as protein is also sensitive to thermal and photolytic treatment during the synthesis process hence might affect their molecules. Furthermore, large molecules of protein and peptides suffer from slow mass transfer kinetic and cannot penetrate the polymer network hence affecting the reoccupation process for rebinding capability (Mahony *et al.*, 2005; Refaat *et al.*, 2019).

### **Conclusion**

In conclusion, MIP offers very promising potential in the development of electrochemical sensor, especially in enzyme-free electrochemical sensors. Current researches demonstrate the progress of MIP chemistry and its contribution in various analytical problems. MIP can be categorized as one of the advanced synthetic methods for designing robust recognition materials that mimic biomolecules such as enzymes and antibodies that have been used in many applications. However, it is also important to understand and address some of the issues regarding MIPs that have been highlighted in this review with the main focus is to improve and optimize their specificity, sensitivity, and stability for effective use in any given analyte. These are crucial as it can be an effort to put this technology in the marketplace against other conventional electrochemical sensors. Therefore, more research in the future is substantial in improving MIP technology especially to be able to bring this technology into the commercialization phase.

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