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The Role of Molecularly Imprinted Polymers in Sensor Technology: Electrochemical, Optical and Piezoelectric Sensor Applications

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Abstract: With the help of molecular imprinting technology, artificial receptors can be made and used for identification. This technique's limitless application increases polymer technology and makes it adaptable to other technologies. In this study, examples of sensor applications are used to explain molecular imprinting technology (MIT) and its brief history. MIT can be used to create polymer-based artificial receptors with remarkable selectivity and affinity to detect any target molecules that can be imprinted on a polymer. A monomer is synthesized around a template molecule to create a selective cavity that serves as an artificial receptor. Molecularly imprinted polymers (MIP) offer a wide range of uses and have recently garnered much attention. These polymers' production methods, production kinds, and molecular imprinting techniques are all thoroughly detailed. The outstanding properties of MIPs make a crucial contribution to sensor applications offering selective, fast, easy, and cost-effective analysis, which became very popular after Clark published his first biosensor study. Apart from the biological recognition receptors, MIPs have the advantage that they are not affected by physical conditions of the environment, such as temperature, pH, and ion strength. To overcome the biological recognition receptors' disadvantages, molecularly imprinted polymers can be used for sensor development. From the point of view of the review, the combination of MIPs and sensors was explained and proposed as an informative paper.

Keywords: Sensor, molecular imprinting, molecularly imprinted sensors, polymerization, electropolymerization.

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1. INTRODUCTION

Recently, it has been exciting to follow the development of biosensor technology, especially with its applications in the health field, which has high miniaturization and almost unlimited application potential. The growth rate of these small systems, which are formed by combining a biological molecule with a non-biological element, increases almost exponentially. They are interesting because they can develop quickly, their research and development costs are low, and the innovative aspects of the developed technologies are high. On the other hand, one of the most significant advantages of biosensor technology is that it includes interdisciplinary fields.

It can consist of organic chemistry, biochemistry, electrochemistry, nanotechnology, electronics, material chemistry, and many other areas. Like every field, biosensor technology's advantages and disadvantages cause new developments, especially in this field.

The biosensor concept first met the literature with the system used by Clark to measure the amount of oxygen in the blood (1). That design turned into a product and has recently been used as a commercial glucose monitoring device known in the scientific community as glucose biosensors (2–5). From the oxygen-measuring biosensor technology, miniaturized analyzer systems have been produced and developed in different fields with each developed biorecognition agent. Although it is not a specific area, there have been developments in many areas, from health to food, from environmental factors to hazardous chemicals (6). The most critical point in the biosensor system is that it can analyze the target analyte in a specific, selective, and sensitive manner,

as in every analyzer. The biorecognition receptor element in the biosensor technology, which contains these most important features, provides specificity, sensitivity, and selectivity for target molecule detection. Since it is known that biosensors consist of a transmitter and a biorecognition receptor, they are systems that generally work by monitoring the signal created by the interaction of the analyte molecule with the biorecognition receptor immobilized on the transducer. This interaction can monitored by electrochemical, optical, or mass transfer (piezoelectric) based transducers with different interactions (7). All these methods divide into subgroups and transducers used by the biochemical reaction, targeting the interaction of the target molecule and the biorecognition receptor. The most used electrochemical sensors can be subdivided into different classes as amperometric (current-based), potentiometric (voltage-based), impedimetric (resistance-based), and capacitance (double layer charges based) measurement due to the interactions between the selective layer and the target molecule on the transducer (electrode) surface (8).

On the other hand, optical sensors are based on the measurement of absorbance, fluorescence, chemiluminescence, or surface plasmon resonance. In optical systems, measurement is carried out with an optical transducer; on the contrary, the detection is different in surface plasmon resonance (SPR) systems. Here, the reflection of a laser transmitted on a chip is changed over time by the biorecognition receptor and analyte interacting on the other side of that chip. SPR systems are based on the fact that the interaction of the receptor with the analyte changes the dielectric constant of the chip. Piezoelectric systems, which are mass-based measurement systems, are based on the principle of changing the resonance in piezoelectric crystals with the interaction of the receptor and the analyte. Based on all systems, the analog signal is released by the reaction of the biorecognition receptor with the analyte detection by the transducer and converted into a digital signal (9). The combustion agent that creates the analog signal can be different biomolecules. As described in the previous sections, these biomolecules can interact with the catalytic or affinity-based analyte molecule. Catalytic biomolecules can be enzymes, whereas affinity-based ones can be molecules that do not react with the analyte, such as antibodies, DNA, and cell surface receptors that can interact with each other in a specific way (10).

Although biological receptors (enzyme, DNA, antibody) used in biosensor systems offer high sensitivity measurement due to their high cost, special storage conditions, and optimum operating conditions (such as pH and temperature), their use is limited (11). Due to this restriction, new physical-based recognition receptors are being developed. This technology, which is seen as a technology to create similar biorecognition receptors, is called molecular imprinting technology (MIT). Simply called, it is the name given to the polymerization of a monomer in a certain way. Molecularly imprinted polymers (MIPs) have been seen as a solution to the usage limitations of biorecognition systems since they do not require special physical conditions such as temperature, pH, or ionic strength optimization required by the receptors used in biosensors. A broad spectrum of solutions is offered in this technology, which has not only sensor technology but also different application areas. Molecularly imprinted polymers have applications in different areas, such as solid phase extraction (11), affinity chromatography (12), controlled drug release (13), catalysis(14), and chemical/biochemical sensors (15). Although they are primarily used in affinity-based separation applications, their use in sensor systems has increased recently (16).

2. MOLECULAR IMPRINTING TECHNOLOGY

Molecular imprinting technology (MIT) can be explained in the most general sensor technology as the technique of creating artificial receptors containing specific cavities around the target molecule to be analyzed, and polymeric structures created with this technology can find applications in a wide range of industries, from food to health (17). In other words, when these polymers, developed as synthetic, smart receptor technology, are used in biosensors, they are called sensors because synthetic receptors are used instead of biorecognition receptors. Because the recognition receptor of biological origin leaves the system. In this way, a more resistive element of the system makes it extremely easy to use, although it has disadvantages in some usages. Molecular imprinting technology allows the production of synthetic polymers that mimic the antigen-antibody relationship in nature. These polymers enable new measurement systems with the sensitivity and selectivity of biological sensors but are unaffected by the harsh conditions in which they are affected (18). Molecularly imprinted polymers have been prepared by the copolymerization of the desired molecule (target), one or more monomers containing appropriate functional groups, and crosslinking chemicals in a suitable solvent environment (Figure 1). After removing the target molecule from the synthesized polymer, gaps are formed similar to the target molecule in shape, size, and chemical composition. By reattaching the target molecule to these gaps, systems allowing selective, sensitive, and rapid determination of the target molecule can be prepared (19). M. V. Polyakov is credited with creating the first instance of the idea of molecular imprinting in 1931 for his work on polymerization with sodium silicate and ammonium carbonate utilizing a novel synthesis method (20). However, the concept of the imprinted polymer was first used by K. Mosbach and B. Sellergren (21) in a study published in 1984 - the first study using the imprinted polymer definition, written by G. Wulff (22), was published in 1985, although Wulff had been publishing a series of articles since 1973, entitled "Polymers of Enzyme-Analog Structure" (16). Both of these researchers are working on molecular imprinting technology, but they have focused on different methods in polymer synthesis: Wulf has used covalent bond-based synthesis methods, while Mosbach has prepared polymers with

non-covalent interactions-based methods. The significant difference between the two methods is the desorption process, defined as releasing the analyte from the polymer. Although covalent imprinting offers a very stable and homogeneous binding environment, the non-covalent method is considered more valuable because of the difficulties in the desorption process of covalent imprinting (23).

Non-covalent procedures are considered to be more technically convenient when compared to covalent ones. In this context, separating the imprinted molecule is comparatively more straightforward compared to the covalent bond. A notable benefit of the proposed methodology is the substantially reduced detrimental impact on the polymer during the non-covalent separation process. Consequently, this contributes to an extended lifespan of the molecularly imprinted polymer (MIP). Furthermore, the enhancement of repeatability is a highly desirable characteristic often sought in sensor technology. The interaction between the analyte and polymer is contingent upon the characteristics of the cavity. However, it is also desirable for the overall structure of the polymer to remain unchanged. The ability to customize the design of this cavity further enhances its selectivity. Although a cavity in the form of a target molecule is produced, the properties of the chemical parts in this cavity are also found in the non-imprinted parts of the polymer. This usually causes non-specific binding. This is a handicap in large molecules. This handicap causes a false-positive signal, especially in susceptible systems such as EIS, SPR, or QCM. Different imprinting techniques have been used to overcome this situation. The most popular type of molecular imprinting, epitope imprinting, improves selectivity in molecularly imprinted polymers. Therefore, MIPs offer a wide area of use, especially in sensor technology, with features like high selectivity, durability, long-term stability, and easy preparation. Epitope imprinting provides the opportunity to replace the antibodies used in biosensors and is called artificial biomimetic antibodies (7).

Molecular recognition reactions are one of the most essential elements of biological mechanisms. Molecularly imprinted polymers are designed to mimic molecular recognition elements in nature. Molecular recognition often includes non-covalent interactions such as van der Waals forces, ionic interactions, dipole-dipole interactions, pi bonds, and hydrogen bonds. Molecular recognition is based on the key-lock relationship between the enzyme and substrate. Molecularly imprinted polymers are also specific to the template molecule on which they are based, just as a key is specific to a lock. The molecular imprinting technique is used to produce polymer materials that mimic the structure and properties of a particular target molecule. This target molecule is used as a template during the imprinting process. Just as a key fits into a particular lock, molecularly imprinted polymers are designed to have the ability to recognize a particular target molecule. The template molecule forms the basis of this recognition feature and creates a specific shape and structure within the polymer material. This shape and structure provide similarity between the template molecule and the target molecule so that the polymer material can selectively capture the target molecule. A different template molecule must be designed for each target molecule because each has its unique structure and properties. Thanks to this design, the polymer material can recognize and select the target molecule; similarly, a key fits only in a suitable lock (24). Figure 1 shows a schematic summary of recognition by molecularly imprinted polymers.

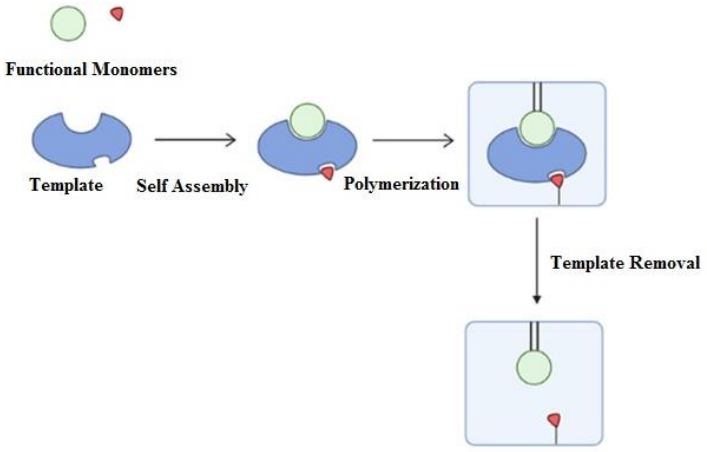

Figure 1: The principle of molecular imprinting recognition.

2.1. MIP Synthesizing Methods

In polymers produced with molecular imprinting technology, the first thing to consider is the physical structure of the target molecule. Since this structure will be directly related to the functional groups on the monomer, the biggest handicap lies here. When an analyte-containing multi-functional group, e.g., protein, is selected, one monomer may not be sufficient. The presence of a homopolymer can reduce selectivity. Therefore, it is crucial to turn to copolymers. Problems with the polymer synthesis process arise as the amount of monomers rises. For this reason, when we look at the sensors developed with molecular printing technology, it can be seen that sensor systems targeting simple molecules have been produced in general.

Universal polymer production reactions are used in the production technique, which is generally a

biomimetic technique. In these systems, if the target is a molecule that can be denatured, some polymerization techniques may experience problems. Because if the polymerization liquid is organic, a biological molecule will be denatured, and the resulting cavity will be shaped to recognize the denatured structure. Considering these, the target analyte should be measured before denaturation.

As we mentioned before, a wide variety of production methods are available for MIP synthesis. The only difference is that the imprinted molecule is added to the mixture. The polymer composition ratio is another critical point except for the target molecule structure (25). After selecting the target to be imprinted, the next step is to select the monomer. This is crucial for the sensor system's functionality since the monomer will grow together with the target molecule's binding. It should also avoid binding unspecific targets.

The target molecule's physical size and the presence of different reactive side groups suggest that selectivity will be a significant challenge. The cross-reactivity in question here may occur with molecules with similar properties. One of the ways to overcome this is to produce multiple but weak interactions. Another critical point is the concentration of the cross-linker. In this case, it is necessary to decrease the concentration of the cross-linker with the increase in the size of the molecule, that is, the target molecule. As a result, the properties of the template molecule and the required monomer have an important place in determining the composition of the polymer.

In MIP synthesis, more than one monomer can be used to increase the selectivity for the target molecule. The monomer-target ratio, on the other hand, is based on preparing a functional monomer group for each chemical bond due to the nature of the polymerization. Experimental conditions optimize this ratio, and it is imperative to prevent non-specific binding. The effect on the monomer/target ratio can also be in the form of copolymerization. The creation of steric effects has an essential place in molecular imprinting technology.

By copolymerizing functional monomers and the required target molecule with cross-linker molecules present, MIPs can be created. The bonding herein may be by covalent or non-covalent interactions, depending on the method chosen (26). The functional monomers selected for polymer synthesis are responsible for providing the functional groups required for non-covalent bonding or the reactive substituents required for covalent bonding. Examples of highly preferred monomers are acrylamide, methacrylic acid, pyrrole, and aniline. In addition, conductive functional monomers such as pyrrole, o-phenylenediamine, thiophene, aniline, dopamine, o-aminophenol, and p-amino thiophenol are preferred for the polymerization process by electropolymerization. Acidic and basic monomers with the capacity to make covalent bonds, hydrogen bonds, and other interactions can be used. Polymerization is carried out by adding functional monomer and template molecule to the polymerization medium, as well as cross-linkers such as ethylene glycol dimethacrylate (EGDMA) and tetraethyl orthosilicate (TEOS) (18,27). Most often employed techniques include precipitation polymerization, electropolymerization, and photopolymerization. In some polymerization methods, a chemical may be required to initiate the reaction; hence, Azobisisobutyronitrile (AIBN) and N,N,N',N'-tetramethylethane-1,2-diamine (TEMED) are the most commonly used initiators.

The new technology MIP's is not just a mixture of polymer and template. The use of nanomaterials as polymer composites in sensor technology has increased in recent years due to their properties, such as increasing the surface area and having high conductivity (28). With these advantages that nanomaterials add to sensor technology, sensor technology has advanced. They are very useful in reducing the potential required for electropolymerization, especially in studies where electrical conductivity is effective on polymerization. High surface area increases the quality of the polymer formed. Nanomaterial-modified polymers, which are generally very useful in terms of forming a thin layer to reduce limitations, also eliminate many disadvantages in sensor technology.

MIPs have several disadvantages, such as containing target molecule residues, limited selectivity, and limited mass-carrying ability. Various methods have been used to overcome these disadvantages: the thin film polymerization method has been used on the electrode surface to increase mass transport, and nanomaterials have been used to increase the surface stabilization and sensitivity of the method by increasing the surface area. In addition, nanomaterials such as carbon nanotubes, graphene, and fullerene help to increase reusability and surface conductivity (29). Additionally, through the reaction between both the polymer and the template molecule, imprinting, polymerization, and manufacturing can all be accomplished using imprinting procedures. This interaction can be covalent, non-covalent or semi-covalent (30). A covalent link is created between the functional monomer and the template in the covalent imprinting technique. With this bond, the template molecule is chemically modified to form this bond before monomer-pattern formation. The covalent bond on the polymer produced as a result of this modification is broken, and the template is eliminated. This removal causes covalent bonds to rebuild, contri-buting to backlinking's selectivity. This method is called the pre-organized approach.

The non-covalent method is based on the self-assembly principle. There will be non-covalent interactions active here. These are hydrogen bonds between donor and acceptor, electrostatic/ hydrophobic interactions, and Van der Waals forces. Imprinting is performed with these interactions. An important point in this technique is that the interaction between the functional monomer and the template that will interact should be strong. Another method is the semi-covalent interaction. In this

instance, the functional monomer is covalently linked to the target, the template. After polymerization, the covalent bond does not re-form, unlike the covalent approach, but the interaction occurs with non-covalent bonds.

Among these imprinting methods, the covalent method is believed to be more effective and provides more homogeneous imprinting. Its stability is also higher than non-covalent methods. The most significant handicap is the step of designing the binding sites of the covalently imprinted molecule. In addition, extreme care must be taken to avoid breaking the covalent bond and damaging the polymer. In contrast, non-covalent imprinting is simpler than covalent imprinting. It is one step because there is a direct polymerization between the template and the monomer molecule. Heterogeneity is higher in contrast to covalent modification.

MIP-based sensor systems must have an appropriate interaction between the recognition element and the transducing surface. The process considered while producing the polymer is how the template molecule to be measured is desired to be stamped. If polymerization on the transducer surface is not suitable, external polymerization is more suitable if electrical polymerization is possible on the electrode surface. In this case, it should be taken into account that the template molecule is imprinted on the transducer surface without losing its solubility and natural structure. The success of the polymerization must be determined by proving that there is no loss of activity with a validated method.

There are two approaches to the preparation of these systems. First, in situ polymerization prepares the recognition element on the conductive surface. Conversely, the prepared MIP is coated on the conductive surface (ex-situ) with a suitable method.

2.1.1. Ex-Situ Polymerization

Ex-situ synthesis methods for MIPs refer to methods in which the polymer material is produced ex-situ under laboratory conditions to develop proper recognition properties on the target molecule. Ex-situ polymerization means polymer synthesized externally, in other words, not on the transducer surface. The polymerization reaction requires a monomer and a chemical initiator that can be a chemical substance. In this way, it can be synthesized in large quantities, stored, and used consistently. It is not necessary to produce in every usage. In this type of polymerization, the polymers to be synthesized are prepared directly in a container without being treated with the support material. They are advantageous because they can be prepared at high temperatures as desired. High temperatures are not feasible for protein or biological element imprinting because denaturation can be problematic.

Additionally, from the perspective of sensor technology, polymer sizes and shapes can be changed in a controlled manner. The most significant handicap here is placing these polymers on the transducer surface, which is the support material. While it is easy to place on large surfaces such as SPR chips, on the other hand, it is quite challenging to fix polymers on the surfaces of microelectrodes. Even if successful, the homogeneous distribution of polymers may affect sensor applications, but when it is achieved, extremely durable and reproducible signals can be obtained. The most significant advantage of polymerization is that the monomer and initiator molecules can be prepared in high volume, and the template molecule can show good distribution. The polymerization components that are well dispersed in the solution successfully produce a homogeneous polymer (31).

Bulk polymerization (32), suspension polymerization (33), emulsion polymerization (34), and surface imprinted molecular imprinting (35) can be given as an example of ex-situ polymerization types. In bulk polymerization, monomers and basic template molecules are combined in a solution or mixture. Then, polymerization is carried out using polymerization initiators. As a result of this process, polymer material is obtained. The polymer material contains cavities with recognition properties around the target molecule in this method. Suspension polymerization is a polymer production method in which insoluble monomers are dispersed in solid particles, and polymer particles are obtained as a result of polymerization of these monomers. In this method, since the solubility of the monomers is low, the monomers are dispersed in suspension in the solid carrier. Emulsion polymerization is frequently used to produce latex paint and latex rubber products. Since the polymer particles produced by this method are dispersed in the liquid in the form of small droplets, the properties and usage areas of the final product can be controlled to a great extent. However, the complexity of the method and the need for careful control of the process parameters can present some challenges that need to be optimized. The surface-printed molecular imprinting method uses a substrate with the target molecule on the surface. Polymerization is carried out around the target molecules on the surface. This method develops specific recognition properties for target molecules on the surface.

Examples of free radical initiators used in polymerization are peroxides, azo compounds (Azobisisobutyronitrile (AIBN)), and persulfates (Sodium, ammonium, or potassium persulfate) (36).

2.1.2. In-situ Polymerization

Another molecularly imprinted polymer preparation process is in-situ polymerization. In this approach, the transducer serves as the surface on which the polymer imprinting process is conducted (37). Same as in ex-situ polymerization, a monomer, template molecule, and an initiator are needed on this support material. The initiator can also be a chemical component or a direct electric current. Because functional semiconductors that can be polymerized electrically can be used today. With these methods, polymerization, that imprinting, can be facilitated. The handicap of the method is that it has to be regenerated each time. This reduces repeatability. However, the consumption of the substance reduces the suppression of the proteins obtained in small

amounts. Another advantage is that if the amount of target molecule is low, the polymer can be produced on this structure by covalent or non-covalent immobilization of the molecule directly onto the transducer surface. This facilitates the imprinting of particularly large proteins or cells. In this way, a large amount of material can be imprinted with a small amount of monomer.

Moreover, in situ polymerization is synthesized on the support material by direct polymerization on the transducer. In this way, sensor production can be carried out directly. The most commonly used technique in polymerization is electropolymerization. Here, the electric current, the electron, is used as an initiator. In this way, rapid polymerization can be achieved.

In addition to electrochemical polymerization, it is crucial to exercise caution when conducting polymerization on undesired regions, particularly the transducer. Failure to do so may result in the generation of false positive signals due to polymerization occurring outside the working electrode. The sensor is ready for usage after direct polymerization on the transducer. There is no retention problem of polymers produced on the transducer. Reproducible production and measurements are therefore possible. In situ polymerization is more effective, especially when the template molecule needs to be imprinted at a low concentration (biomolecule). Although there is no loss of template molecules, high-efficiency suppression is achieved. Efficiency increases with a low amount of monomers and templates (38). In both methods, the imprinting method and the transducer are selected based on the properties of the template molecule.

Each of the molecularly imprinted polymer synthesis methods has several advantages and disadvantages. Their superiority depends entirely on where and for what purpose this polymer will be used. The researcher needs to consider the needs and priorities of the use area when choosing between these methods. When deciding which method is most suitable, factors such as the properties of the target molecule, the properties of the polymer material to be used, the complexity of the manufacturing process, and cost should be considered.

3. MIP SENSORS AND APPLICATIONS

Surface characterization is important after the production of molecularly imprinted polymers. This characterization is carried out primarily with sensor technology, a measurement technique. Secondly, the bonds and interactions between the target analyte and the polymer should be determined by different methods and the adhesion of the polymer to the transducer surface. Bond structures are usually determined by Fourier Transform Infrared Spectroscopy (FTIR). The polymer's bonds with the target molecule should be shown here. The target polymer can then be imaged on the transducer surface after that. The morphological structure can be visualized by examining the transducer surface with an electron or atomic force microscope.

By determining this, optimization processes can be started. In the sensor optimization processes, the polymer binding capacity is determined first. This process is considered by calculating the polymer surface and the concentration of the target molecule. Sensitivity, selectivity, and storage stability tests can be performed, and sensor applications can be started.

Various physicochemical techniques can be applied according to the transducer type to analyze molecules in sensor systems using MIP instead of biological molecules as biorecognition receptors. Optimization of physicochemical techniques is crucial to preserve the durability of polymers. For example, when electropolymerization is performed, applying excessive current may cause excessive polymer oxidation, creating undesirable structures and rendering the imprinting process useless.

The stability of the imprinted polymers with the sensitivity of the sensors and their usability under all conditions increase the commercialization potential of these systems. Therefore, the interest in these systems is increasing day by day. In Figure 2, the Scopus data obtained by scanning "molecular imprinting" and sensor illustrate this increasing interest. MIP-based sensors can be used in many areas, such as the environment, food, and biomedicine, by differentiating the targeted analyte (39). The template molecule can be a small ion-molecule or a macromolecule, such as enzymes and proteins (40). In Figure 3, the working principle of a MIP-based sensor is schematized. With the change of the analyte, the analytical signal and, therefore, the measurement method can also vary widely. These methods can be examined under 3 headings: electrochemical, optical, and piezoelectric methods (41).

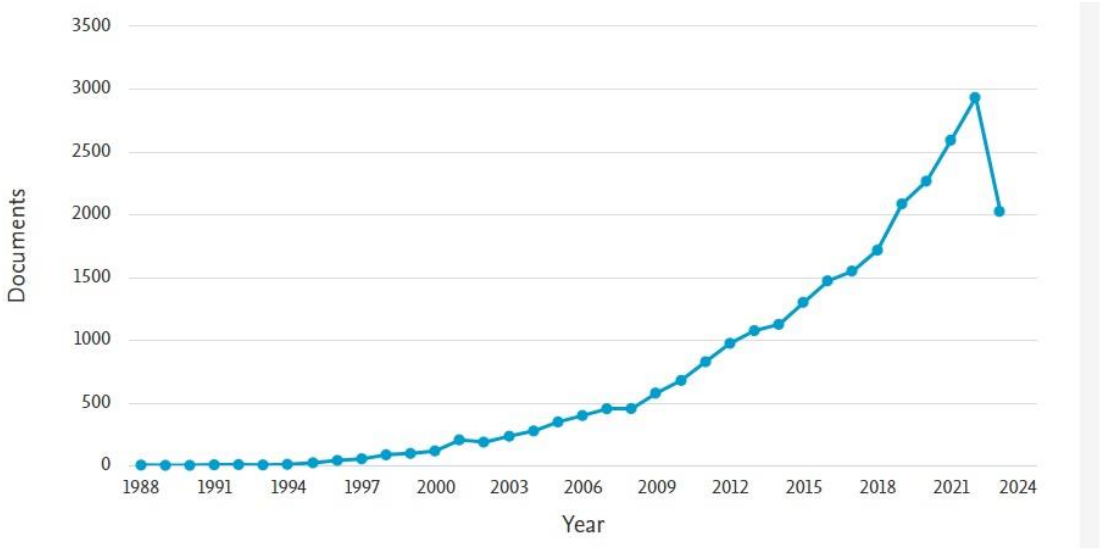

Figure 2: The Scopus data showing articles published from 1988-2023 on MIP-based sensors.

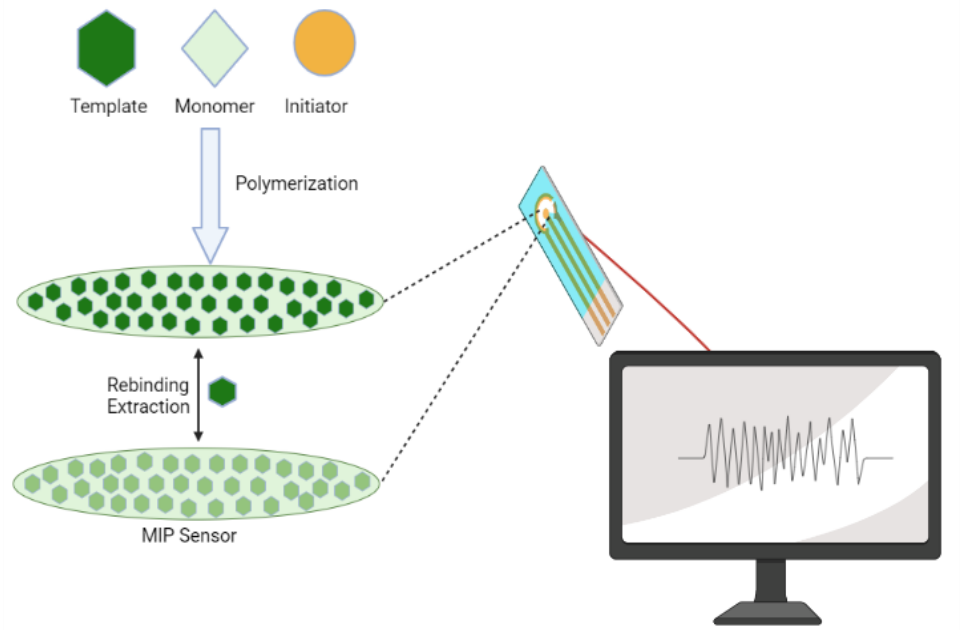

Figure 3: The modification principle of a MIP based sensor.

As we have mentioned before, molecular imprinting technology is carried out using existing polymerization techniques. The most well-known of these techniques is the Bulk imprinting method. Here, the target molecule is directly imprinted into the entire polymer matrix without needing a specific region. The polymer is then mechanically broken down, removing the mold (42). It is preferred for small molecules that are small and will not degrade from mechanical degradation. Apart from imprinting on the entire polymer, only surface imprinting can be performed. Here, the target creates high affinity. Target molecule-polymer interaction is easier because the cavities are not embedded (43). Therefore, it is the ideal method for imprinting large molecules such as proteins. Although this method has the disadvantage of sensitivity to bulk polymerization in itself, it is widely used with different method designs. Among the surface imprinting methods, the soft lithography method, in other words, stamping, can be used on sensor surfaces (44,45). The polymer can be produced by forming a mold with UV curing. Assistance is provided with support solver support. Performing two-dimensional imprinting instead of 3D imprinting allows the development of micro or nano-size polymers (46). Another surface imprinting method is the template immobilization method (47). Here, self-assembling polymerization is performed on the target molecule.

In this way, immobilization of large biomolecules over multiple groups is possible. Another method is emulsion polymerization.

Covalent bonds are used in this instance to imprint the surface. Selective microspheres with a large surface area can be created during the manufacture of spherical polymers by polymerization on a substance in the center (48). Instead of imprinting

the entire molecule in polymerization techniques, the epitope imprinting method is carried out over a portion from which a domain is specifically selected (49). The epitope imprinting method allows for using a tiny fragment or portion of a macromolecule to produce stronger and more precise connections. As a result, affinity can be raised while non-specific binding is reduced. When the sequence is revealed, the imprinted polymer can recognize both the template and the protein. In this way, imprinting can be done with different methods, especially considering the target molecule. It can form a film of the desired thickness in a simple, fast, and low-cost way to prepare MIP by electropolymerization (50).

3.1. Electrochemical MIP Sensors

The engagement of analytes with receptors on an electrode surface can be converted into an analytical signal by electrochemical sensors, detectors with electrochemical transducers. The working electrode, the reference electrode, and the counter electrode are all present in these systems. Gold electrodes and glassy carbon electrodes are preferred as working electrodes. In addition, disposable screen-printed electrodes are also widely used in MIP sensors. Differential pulse voltammetry (DPV), Cyclic voltammetry (CV), amperometry, and electrochemical impedance spectroscopy (EIS) are the most widely used electrochemical measurement methods. These methods are preferred because of their low cost and easy preparation (51). Electrochemical-based MIP sensors are used in areas such as environmental analysis and food and medical applications. The most important point of the electrochemical-based sensor technology is that it can detect molecules in any kind of sample structure. Table 1 provides examples of several MIP-based electrochemical sensors.

In their study, Sundhoro et al. devised a sensor to facilitate the monitoring of allergens present in soy-based food products. The MIP-modified sensor has the capability to detect food allergens at a concentration of 100 parts per billion (ppb). The basis of the method is electrochemical surface investigation techniques such as CV and EIS. The method was also compared with food allergen lateral flow tests. In this study, genistein-specific cavities were formed by electropolymerization of o-phenylenediamine dihydrochloride at 1.2 V in one minute. Imprinted occurred via N-H bonds. However, this study is an analyte-centered redox method, therefore, quercetin and 7-hydroxy flavone redox potentials were used to detect food allergens on the screen-printed electrode in the presence of the genistein. The method was based on the non-covalent technique and performed successfully (52). Aghoutane et al. prepared a sensor based on electrochemical impedance spectroscopy for the determination of malathion (MAL), an organophosphate pesticide in olive oil and olives. The imprinting was carried out via the N group of the MAL, and the MIP formation was carried out by using TEMED, acrylamide, and ammonium persulfate as initiators including MAL detection was carried out electrochemically by CV, EIS, and DPV methods were also used for the electrochemical characterization of the surface. The detection limit was calculated as 0.06 pg/mL, and the calibration curve was between 0.1 to 1000 pg/mL (53). Ayankojo et al. prepared a MIP-based sensor for monitoring the antibiotic erythromycin in water. The erythromycin selective MIP surface was prepared by polymerizing m-phenylenediamine (mPD) on the electrode surface and analyzed by cyclic voltammetry. The limit of detection (LOD) was calculated as 0.1 M (54). Mazouz et al. developed a sensor for prostate-specific antigen (PSA) detection by electropolymerization of pyrrole. The basis of the imprinting is NH groups hydrogen bond formation to LOD protein amino acid residues. The MIP. formation was observed by chronoamperometric technique and square wave voltammetry for PSA analysis in the study. It is calculated by Hill equation $K_d = (1.02 \pm 0.54) \times 10^{-14}$ M. The method's applicability was examined by contrasting it with ELISA utilizing actual serum samples (55).

Table 1: Electrochemical sensors with performance parameters.

Method	Monomer	Target Molecule	L.O.D.	Ref.
DPV	mPD	ncovNP	27 fM	(56)
SWV	Acrylic acid	Tetracycline	1.5×10^{-7}mol/L	(57)
DPV	Pyrrole	5-HIAA	15×10^{-12} M	(58)
EIS	APBA-Pyrrole	Glucose	6.064 mg/dL	(59)
CV, EIS	*o*-PD	Trypsin	70.9-ng/mL	(60)
Amperometry	MAA	Tetracycline	0.026 mg/L	(61)
SWV	MAA-DVB	2-furaldehyde	$4.67x10^{-5}$ M	(62)
EIS	Acrylamide	Cortisol	0.14 nM	(38)
EIS	oPD	SARS-CoV-2-RBD	0.7 pg m/L	(63)
SWV	Pyrrole	PSA	n.d	(55)
SWV	MAA	Melamine	$1.75*10^{12}$mol/L	(64)
DPV	MAA	Milk amyloid A	5 pg/mL	(65)
DPV	Pyrrole	Troponin T	0.006 ng/mL	(66)
EIS	PVC COOH	Myoglobin	2.25 µg/mL	(67)
DPV	oPD	TGF-β1	0.09 ng/mL	(68)
DPV and EIS	Acrylamide	Tofacitinib citrate	3.48×10^{-13} M	(69)
DPV	o–PD	Amyloid-β(1–42)	0.018 ng/mL	(70)
DPV	Itaconic acid	Metribuzin	0.1 pg/mL	(71)
Potentiometric	Polyaniline	Ketamine	1.0×10^{-6} M	(72)

3.2. Optical MIP Sensors

From the perspective of matter-light interactions, optically active molecular imprinted polymers are used to develop optical sensor technologies. Surface plasmon resonance is the most widely used technique among optical systems (73). This technique works by changing the laser's reflection angle projected onto a gold chip. This reflection angle is realized by the modifications made on the gold chip. In addition, MIPs interferometry used in optical sensor technology can be used with Reflectometric Interference Spectroscopy (RIfS) (74) and Surface-enhanced Raman spectroscopy (SERS) (75) techniques. Most MIP-based sensors in the literature are optical methods such as surface plasmon resonance based on refractive index change (23). These sensors convert optical property changes on the electrode surface into readable signals. Optical sensor systems can be examined in two classes (76).

Affinity-based optical systems, which assess changes in optical properties like fluorescence, absorbance, and refractive index, are the first class. Surface plasmon resonance (SPR), which is dependent on the measurement of refraction of light, is the method that falls into this class and is frequently used in the literature (77,78). Second-class optical sensors are called optoelectronic sensors. The usage of monomers that can choose the optical property change on the surface forms the basis of these systems. These monomers are expected to have high quantum yields and molar absorption coefficients (76).

Application areas of MIP-based optical sensors can be listed as disease diagnosis, food analysis, and environmental pollution monitoring (See Table 2) (50).

A nanoparticle-based optical sensor for pharmaceutical monitoring was created by Altintas et al. The synthesized MIP nanoparticles, whose dimensions were determined as ~132.3 nm ± 3.2, were immobilized on the SPR gold sensor with EDC/NHS. The prepared sensor was used to monitor diclofenac in water and was verified by the LC-MS method. A study of the kinetic data revealed that a dissociation constant of 1.48×10^{-9} M was attained. (79). Motaharian et al. created an optical sensor for detecting the antibiotic sulfasalazine (SSZ) (MIP-SG) by combining molecular imprinting with sol-gel methods. The UV-Vis spectroscopy technique was used to determine the binding characteristics of the sulfasalazine sensor. The 0.26 g/mL detection limit was chosen because of its high reproducibility (RSD = 4.1%) (80). An optical sensor device based on fluorescence was created by Chen et al. to measure anthracene. Bisphenol A and p,p'-diiso-cyanato-diphenylmethane were chosen as monomers, and trihydroxybenzene, p,o,p'-triisocyanatodi-phenyl-methane as cross-linkers for MIP synthesis (81). Sanguanprang et al. prepared an Opto sensor to determine thiamphenicol using a mesoporous and quantum dot hybrid fluorescence sensing probe with molecularly imprinted polymer technology. Mesoporous carbon and CdTe*CdS*ZnS quantum dots were trapped inside the molecularly imprinted polymer to create the hybrid probe. The prepared probe was characterized by SEM, TEM, and particle size analyses, and all the conditions for determining thiamphenicol were optimized. The thiamphenicol analysis is determined based on the fluorescence quenching of the hybrid nanostructure by thiamphenicol. The linear detection range of the system is between 0.10 and 100 µg/L with a coefficient of determination (R^2) of 0.9979, and the detection limit is 0.04 µg/L. The developed optosensor's accuracy was assessed by assessing the spiked milk samples, and the results were compared to those obtained using high-performance liquid chromatography (HPLC) analysis. The recoveries were determined in the range of 93.5% to 100.1% with high sensitivity (R.S.D. < 5%) (82). Chullasat and colleagues created a sensor system for the selective and sensitive monitoring of amoxicillin using molecularly imprinted polymers (MIP-QDs) coated on CdTe quantum dots as photoluminescence probes. MIP-QDs were prepared by the sol-gel method using 3-aminopropylethoxysilane as the functional monomer, tetraethoxysilane as the cross-linker, and amoxicillin as a template molecule Following the desorption of the target chemical from the polymer using ethanol as the desorption agent, amoxicillin-specific cavities in MIP-QDs were produced. The functional groups of amoxicillin and the amino group of the 3-aminopropylethoxysilane chosen as a functional monomer formed a hydrogen bond, and the cavity's size and shape helped to give good selectivity. Amoxicillin more effectively quenched the photoluminescence of MIP-QDs than NIP-QDs, the unimprinted polymer made without the template molecule. Under ideal circumstances, it was discovered that the system's linear detection range and detection limit were 0.20-50.0 g/L and 0.14 g/L, respectively. With a relative standard deviation of less than 6%, it can be said that the system has strong repeatability and reproducibility. The developed method determined amoxicillin in egg, milk, and honey samples, and a recovery of 85-102% was obtained (83). Yu et al. prepared molecularly imprinted phase change microcapsules embedded in carbon quantum dots (CQDs) and developed an innovative fluorescence detection system (MIP@CQDPCM) to determine tetracycline under high-temperature conditions. The system was made ready by microencapsulation of n-eicosan as a phase change material in a SiO shell embedded in CQDs. A molecularly imprinted polymer with cavities specific to this molecule was obtained by removing tetracycline from this polymeric structure. This prepared structure was characterized by SEM, TEM, and fluorescence microscope imaging analysis. Observing fluorescence quenching by binding the tetracycline molecule to the MIP structure, it was seen that the system worked successfully. A sample application of the MIP@CQD-PCM structure was done with local water samples, and the results showed high selectivity and good reusability (84).

Table 2: Optical sensors with performance parameters.

Method	Monomer	Target Molecule	L.O.D.	Ref.
SPR	Methacrylic acid	Ciprofloxacin	9.71 nM	(85)
SPR	Multi monomer	Secreted bacterial factor	0.23 nM	(86)
SPR	2-methacryloyloxy ethyl phosphorylcholine	Cortisol	4.8 pM	(87)
SPR	Graphene-Dopamine	L-Tryptophan	0.105 mM	(88)
SPR	PGA	Kanamycin	12 nM	(89)
SPR	NanoMIP	Casein	127 ng/mL	(90)
RIfS	Methacrylamide	PenG	4.32 mM	(91)
Reflectance spectra	IIP-AMPSA	Pb(II)	85 ng/L	(92)
Fluorescence	a-CQDs	Tannic acid	0.6 nmol/L	(93)
Fluorescence	PANV-GMA	Paracetamol	1.00×10^{-6} M	(94)
Fluorescence	AA, MBAA, AMPSA, DEAEM, and allylamine	Aflatoxin B1	20 ng/mL	(95)
SERS	4-MBA and 4-VP	Patulin	5.37×10^{-12} M	(96)
SPR	Methacrylic acid	Adenosine	0.018 M	(97)
SPR–LMR	NanoMIP	Transferrin (HTR)	13.6 fM	(98)
SPR	Methacryloylamidoglutamicacid (MAGA)	Zearalenone	0.33 ng/L	(99)
SPR	N-methacryloyl-L-phenylalanine	Benzo[a]Pyrene	14.97 ng/L	(100)
SPR-POF	Acrylamide	Glyphosate	0.04 µg/L	(101)

3.3. Piezoelectric MIP Sensors

The piezoelectric effect can be described in its simplest form by the voltage applied to a material, creating a wave in the crystal. The potential applied to the piezoelectric material's surface creates vibrations in the substance. Crystals without a center of symmetry are piezoelectric materials, such as quartz (SiO_2), aluminum phosphate (berlinite), potassium sodium tartrate tetrahydrate (Rochelle salt), and polyvinylidene fluoride (102). Piezoelectric measurements are preferred methods, especially in affinity-based biosensor applications, because they can be analyzed without any label application (such as fluorescence or chemiluminescence).

This approach is based on detecting the oscillation with the mass attached to the surface of the electrodes on the piezoelectric crystal, which is a part of the sensor system, and the measurement of this change (78). Piezoelectric sensors have become popular in recent years with their simplicity, ease of use, low cost, and measurement speed, among other methods that do not require labels. Quartz crystal microbalance (QCM) is one of the most commonly used piezoelectric measurement methods (Table 3). Quartz microbalance is preferred for mass-sensitive measurements and chemical analysis (103). Another type of piezoelectric sensor system is the surface acoustic wave method. Acoustic wave sensors can vary in their use of different vibration levels and wave modes. In sensors of all classes, physical changes in the material in contact with the wave propagation level can be monitored in real-time. Current research using these sensors focuses on measuring samples and their molecular types and concentrations.

Table 3: QCM-based sensors with performance parameters.

Method	Monomer	Target Molecule	L.O.D.	Ref.
QCM	Pyrrole	Trichloroacetic acid	1.0 µg/L	(104)
QCM	MAA	Methimazole	0.109 mg/L	(105)
QCM	Methacryloylamido tryptophan	Bilirubin	0.45 µg/mL	(106)
QCM	MAA	Methomyl	n.d.	(107)
QCM	MIP Monolayer	Thiacloprid	10 µM	(108)
QCM	Methacrylic acid	Formaldehyde	500 ppb	(109)
QCM	mPD	Amoxicillin	0.2 nM	(110)
QCM	3-TAA	Melphalan	5.40 ng/mL	(111)
QCM	Methacrylic acid	L-tryptophan	0.73 ng/mL	(112)
E.QCM	*p*-Phenylene diamine	S-cathinone	0.12 ng/mL	(113)
QCM	MAGA	Chlorpyrifos	$3.0\ 10^{-13}$ M	(114)
QCM	MAGA	Zearalenone	0.30 ng/L	(115)
QCM	Methacrylic acid	Diethylstilbestrol	2.63 ng/mL	(116)
QCM	Acrylamide	AHL	0.055 ng/mL	(117)
QCM	MAA	*Bactrocera dorsalis*	24.68 mg/m^3	(118)
QCM	PVAc/BA	NH_3	22.9 ppm	(119)

By monitoring physical and chemical changes, these sensors can be used in fields such as medicine, food, and environmental sciences. Two sensing principles exist in such systems: the first is when the voltage field is affected, and the other is when the piezoelectric field (potential) is affected (120). In the study of Ermolaeva et al., molecularly imprinted polymers synthesized by the precipitation method were used to prepare sensors for Ractopamine determination. Methacrylic acid was chosen as the monomer for MIP synthesis. Cyanoacrylate was used to immobilize the produced nanoparticles on the electrode surface. MIP and cyanoacrylate solution were dropped on the piezoelectric crystal, and the spin coating method was used. The study's detection limit was discovered to be 12 g/mL (121). To detect terpinyl acetate in small doses, Debabhuti et al. created a QCM gas sensor (aTA-MIP-QCM) by covering the quartz crystal with a polymer containing polymethacrylic acid (PMAA). The limit of detection was calculated as 4.46 ppm (122).

4. CONCLUSION AND FUTURE REMARKS

Molecular imprinting, which is less or almost unaffected by physical conditions, creates the sensors of the future, where artificial receptors can be created. Molecular imprinting technology has been developed with artificial receptor logic in determining different analytes, and sensor applications are also pervasive. Among its advantages, reducing measurement costs is the most significant factor. While providing this, time-saving in the production phase, easy reproduction, and low cost of consumables used to play a crucial role. Thanks to the advantage of overcoming the problem of denaturation, which is the most significant handicap of biosensor technology, it can be easily predicted that sensor technologies that can increase shelf life will be available soon. The fact that the optimum working conditions, essential for biological receptors, are not sought for performance shows the system's usefulness. Although the selectivity as the selectivity of a biological molecule has not been achieved, the sensor as a promising technology attracts the attention of scientists.

Another advantage is the regeneration potential of molecularly imprinted polymers unaffected by physical conditions. This stage reduces the measurement costs.

For example, biomolecules such as cell surface receptors are extremely sensitive to external factors used in biosensor systems and have a low regeneration potential. The reason for this is the tendency of the method applied for regeneration to disrupt the structure of this biomolecule due to its high affinity for the target molecule. At this stage, MIPs stand out, especially with their regeneration potential. The inability to regenerate some biorecognition receptors also makes using MIPs interesting. In addition, it can be said as another advantage that optimum conditions are required for the biorecognition receptor to act, but these are eliminated in the use of MIP. Developments in this technology will enable the development of technologies such as enzymes that can dynamically change according to the target molecule or technologies where more than one monomer can be produced in a controlled manner. The engineering of more than one monomer remains the biggest challenge. This is an obstacle to overcome in imprinting high-structure target molecules. When this obstacle is removed, it is inevitable to develop more futuristic technologies.

The introduction of monomers, copolymers, and additional monomers leads to the formation of cavities, subsequently enhancing the structure's selectivity. This augmentation will be feasible through the utilization of cross-linkers or materials that possess the capability to generate diverse composites. The utilization of nanoparticles in polymer technology has the potential to enhance sensitivity and selectivity due to their increased surface area. This phenomenon expands the boundaries of its programmable capacity, enhancing molecular imprinting technology's utility. The fast evolution observed in the characteristics of molecularly imprinted sensors holds great potential. Given the tremendous pace of advancement in this field, one may hypothesize that integrating artificial enzymes onto the surface of sensors may be within close reach.

5. CONFLICT OF INTEREST

Authors declare that they have no conflict of interest in The Role of Molecularly Imprinted Polymers In Sensor Technology: Electrochemical, Optical and Piezoelectric Sensor Applications.

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