



ELECTROCHEMICAL BIOSENSORS BASED ON MOLECULARLY IMPRINTED POLYMERS (MIPS) AND SURFACE PRINTING FOR INFECTIOUS DISEASE DETECTION

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ABSTRACT

Electrochemical biosensors have emerged as promising tools for the diagnosis of infectious diseases due to their high sensitivity, rapid response, and low cost. The use of molecularly imprinted polymers (MIPs) and surface imprinted polymers (SIPs) as robust and selective artificial receptors has gained significant attention. This review summarizes the recognition mechanisms between MIPs/SIPs and various infectious disease biomarkers, including small molecules, microbial toxins, viruses, and bacterial or fungal cells. Different MIP/SIP fabrication techniques such as electropolymerization, coating, and micro-contact imprinting are discussed. The review also highlights clinical applications of MIP/SIP-based biosensors for detecting specific pathogens, such as HPV, HIV, E. coli, and Zika virus. Key challenges such as enhancing sensitivity and selectivity, as well as the future potential of integrating artificial intelligence in biosensor design, are also addressed

Keywords: Electrochemical biosensor, Molecularly imprinted polymer, Infectious disease

INTRODUCTION

Infectious diseases remain a major threat to global health, despite significant advances in treatment and prevention. The World Health Organization (WHO) continues to record high morbidity and mortality rates due to infections caused by viruses, bacteria, fungi, and parasites. Environmental changes, urbanization, and increased global human mobility contribute to the spread of new and existing pathogens. Therefore, early detection and rapid diagnosis systems are crucial for controlling infectious diseases. However, diagnostic methods currently commonly used in clinical laboratories, such as microbial culture, ELISA, and PCR, require extensive time, expensive resources, and require trained

personnel, making them unsuitable for rapid field application or under resource-limited conditions (Fauci & Morens, 2012).

With the need for rapid, easy, and inexpensive diagnostic methods, biosensors are increasingly being developed for infectious disease detection. Biosensors are analytical devices that integrate biological or synthetic receptors with transducers to generate measurable signals upon interaction between the receptor and the target analyte. One promising type of biosensor is the electrochemical biosensor. The main advantages of electrochemical biosensors lie in their high sensitivity, rapid response, small sample volume requirements, and ease of miniaturization, making them highly suitable

for point-of-care testing (POCT) detection (Cui et al., 2019).

A key component in a biosensor system is the receptor, which is responsible for selectivity and affinity for specific targets. In many cases, natural antibodies and enzymes are used as receptors. However, limited biological stability and high production costs hinder mass application. Therefore, attention has shifted to the use of artificial receptors that can provide similar or even better performance under certain conditions. One technology that has developed rapidly in the last decade is molecularly imprinted polymers (MIPs) and surface-imprinted polymers (SIPs), known as stable and economically producible artificial receptors.

MIPs and SIPs function by creating selective cavities in the polymer matrix that are complementary in size, shape, and chemical properties to the target molecule. These cavities enable selective recognition of even complex molecules such as proteins, viruses, and microbial cells. When used in electrochemical biosensor platforms, the interaction between the target and the cavity results in a change in electrical signal (e.g., impedance or current), which can be detected and analyzed for diagnostic purposes. This makes MIPs/SIPs an attractive alternative to conventional antibodies, especially in environments that require resistance to high temperatures, organic solvents, or extreme pH (Yongabi et al., 2018).

Beyond their robustness, another advantage of MIPs and SIPs is their design and production flexibility. MIPs can be designed to recognize a wide variety of targets, ranging from small molecules like metabolites to large entities like viruses and whole cells. Meanwhile, SIPs are particularly useful in applications that require surface printing of large targets, enabling more efficient and rapid template removal than volumetric printing methods. Fabrication techniques such as electropolymerization, spin coating, and micro-contact imprinting have been successfully developed to precisely deposit polymer layers on electrode surfaces (Givanoudi et al., 2020).

In the context of infectious diseases, the ability of MIPs and SIPs to selectively recognize specific biomarkers is crucial. These biomarkers can be signal molecules (e.g., AHLs from bacteria), toxic proteins (such as aflatoxin), viral particles, or even microbial cell walls. By adjusting polymer components and synthesis methods, specific binding sites can be formed for these diverse targets. Accuracy in biomarker recognition will be crucial for the success of biosensors in providing reliable and sensitive diagnostic results (Saylan & others, 2019).

From a clinical application perspective, MIPs/SIPs-based biosensors have great potential for use in rapid diagnostic systems both in healthcare facilities and in remote locations. Several studies have even demonstrated the ability of these biosensors to work directly in biological matrices such as blood, urine, and serum without the need for complex extraction steps. Further developments in the use of nanomaterials and artificial intelligence-based signal processing open up significant opportunities for the integration of these technologies into modern diagnostic systems. Therefore, studies on their working principles, the types of targets they can recognize, and their potential applications in the detection of infectious diseases are essential.

RESEARCH METHOD

Research Tools

This research employed a literature review approach to examine and analyze the development of molecularly imprinted polymer (MIP)-based electrochemical biosensors and surface imprinting techniques in infectious disease detection. The review was conducted systematically across scientific publications from various reputable sources, including ScienceDirect, PubMed, SpringerLink, Scopus, and Google Scholar. Articles searched covered the period from 2013 to 2024, with inclusion criteria being publications discussing the application of MIPs or surface imprinting in the development of electrochemical biosensors for the detection of

pathogens, toxins, or biomarkers of infectious diseases. Keywords used in the literature search included "electrochemical biosensor," "molecularly imprinted polymer," "surface imprinting," and "infectious disease detection."

The obtained data were analyzed qualitatively and descriptively, emphasizing the type of sensor material used, target analyte, molecular recognition mechanism, and sensor performance, such as sensitivity, selectivity, and detection limit. Selected articles were synthesized into thematic narratives and comparative tables to facilitate interpretation and identification of technology trends. The primary data sources are national and international indexed journal articles, scientific proceedings, and open publications available in full. The analysis was conducted without in-depth statistical formulas, with the aim of maintaining a focus on the technical and application aspects of the biosensor

RESULT AND DISCUSSION

Molecularly Imprinted Polymers (MIP) Theory

Molecularly Imprinted Polymers (MIPs) are synthetic polymer materials designed to have specific binding sites for a target molecule. The basic principle of MIPs is the imprinting of a template molecule into the polymer matrix during the synthesis process, which is then removed, leaving a cavity or recognition site that is complementary in size, shape, and chemical properties to the target molecule. This technique is inspired by the recognition mechanisms of biological molecules such as enzymes or antibodies, but with higher stability due to the synthetic base material's resistance to extreme temperatures, varying pH, and organic solvents (França et al., 2013).

The MIP manufacturing process generally consists of three main stages: the formation of an initial complex between the template and functional monomer, polymerization of the complex with the addition of a cross-linker, and removal of the template to create a specific cavity. The interaction between the template and monomer can be covalent, semi-covalent, or non-covalent. Among the three, non-covalent interactions (such as hydrogen bonds,

electrostatics, and hydrophobicity) are most commonly used because they are easily formed and reversible, and are suitable for a wide variety of targets, including small molecules, peptides, and proteins (Chen et al., 2011).

In biosensor systems, MIPs are typically applied as a thin layer covering the surface of a transducer (such as an electrode) and serving as a recognition element. When the target analyte binds to specific cavities formed in the polymer, changes in surface properties (e.g., resistance or capacitance) occur, which are then converted into electrical signals by the transduction system. This response pattern can be quantitatively analyzed to determine the presence and concentration of the analyte in the sample (Fauci & Morens, 2012; Zheng et al., 2024). A major advantage of this approach is that it is label-free, making the process simpler and faster.

The selection of functional monomers is a critical aspect of MIP design because it determines the affinity and selectivity of the printed cavity. Some commonly used monomers include methacrylic acid (MAA), acrylamide, acrylic acid, and 4-vinylpyridine. These monomers interact specifically with the template, forming a stable complex prior to polymerization. Additionally, cross-linkers such as EGDMA (ethylene glycol dimethacrylate) or DVB (divinylbenzene) are used to form a rigid network structure and maintain the integrity of the cavity after template removal.

In recent years, electropolymerization has become a popular method for forming MIPs on electrode surfaces. This technique allows precise control of the thickness and morphology of the polymer layer by adjusting parameters such as voltage, current, and deposition time. Furthermore, this technique is environmentally friendly because it does not require a chemical initiator and can be performed directly in a solution containing the template and functional monomer. The resulting polymer layer can be characterized using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) (Sharma et al., 2015)..

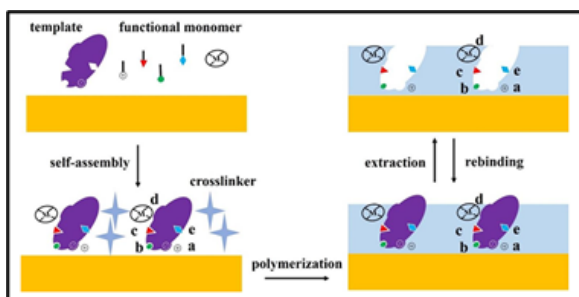


Figure 1. Preparation procedure of molecularly imprinted polymers (MIPs) and surface imprinted polymers (SIPs) on electrodes and various interactions of templates (analytes) and MIPs/SIPs

As a further development, MIPs can also be combined with nanomaterials such as gold nanoparticles, graphene oxide, or carbon nanotubes to enhance sensor performance. This combination can increase the active surface area, electrical conductivity, and binding site density, resulting in significantly higher sensitivity. Furthermore, nanoparticle- or thin-membrane-based MIPs also allow sensor regeneration and repeated use without significant performance loss (Akgönüllü et al., 2020). This approach is particularly well-suited for rapid diagnostic applications in the field.

The main advantages of MIPs over biological receptors are their resistance to extreme conditions, long-term stability, low cost, and the ability to be used in a variety of complex media such as blood, urine, or serum. However, challenges remain, including complete template removal, difficulty in efficiently recognizing large targets, and limited design flexibility for very large and complex biomolecules such as viruses or cells. Therefore, the development of new methods such as surface imprinting and micro-contact imprinting is a potential solution to overcome these limitations (Yongabi et al., 2018).

Types of Biomarkers for Detecting Infectious Diseases

Selecting the right biomarker is one of the most crucial factors in developing MIPs and SIPs-based biosensors. Biomarkers are specific biological indicators used to detect the presence of a disease, including infectious diseases. Biomarkers for infection can range from small molecules, toxic proteins, genetic material, to virus particles or intact microbial cells. With the development of imprinting technology, it is now possible to design recognition cavities in polymers that are compatible with these diverse biomarker types. Each biomarker category presents unique challenges in terms of size, stability, and the chemical interactions required for imprinting and re-recognition.

Small molecules such as metabolites or autoinducer signals from microorganisms are ideal targets for volume-based imprinting. An example is D-arabitol, a specific metabolite of *Candida* spp., which has been used as a template in the development of MIPs for the diagnosis of candidiasis. Under normal conditions, L- and D-arabitol levels are relatively balanced, but during fungal infections, D-arabitol production increases significantly, making it a specific biomarker. MIPs developed using boronic acid monomers have demonstrated selective binding ability to D-arabitol through reversible ester interactions with its hydroxyl group (Dabrowski et al., 2016).

Furthermore, signal molecules such as N-acyl-homoserine lactones (AHLs), which are involved in the quorum sensing system of Gram-negative bacteria, have also been specifically imprinted using magnetic-based MIPs. AHLs are important indicators of virulence regulation, biofilm formation, and antibiotic resistance. Jiang et al. reported the creation of MIPs using methacrylic acid (MAA) as a monomer and AHL analogs as templates, resulting in specific cavities that recognize native molecules through

hydrogen bonding and molecular shape recognition [3]. These MIPs demonstrated high sensitivity and selectivity even in complex matrices such as urine or blood.

For protein biomarkers, such as microbial toxins or viral antigens, challenges arise from their large size and complex tertiary structure. Several approaches have been used to select charged or aromatic monomers capable of forming electrostatic interactions or π - π stacking with protein targets. An example is aflatoxin B1, which was successfully detected using p-aminothiophenol-based MIPs through π -donor-acceptor interactions, resulting in 11-fold greater sensitivity than sensors without imprinting. Similarly, bovine serum albumin (BSA) can be recognized by positively charged MIPs through ionic interactions under physiological pH conditions (M. Jiang & others, 2015).

Viruses as imprinted targets pose challenges due to their large size and structural complexity. However, surface imprinting approaches have enabled the formation of recognition cavities on polymer surfaces that mimic viral morphology. One study demonstrated that Apple Stem Pitting Virus (ASPV) was successfully imprinted onto a polymer aptamer-based hydrogel, where multivalent interactions between the aptamer and viral proteins enabled high sensitivity to the virus. The resulting cavities exhibited a marked volume change upon re-binding of the virus, which can be utilized as signals in optical or electrochemical biosensors.

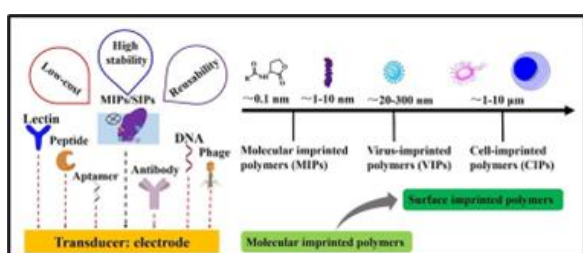


Figure 2. Types of biomarkers in molecularly imprinted polymers (MIPs)

Microorganism cells such as bacteria and fungi can also be directly imprinted using cell imprinting techniques. The characteristic shape and size of cells, as well as the rigidity of their cell walls, allow for the imprinting of their physical topography into polymer matrices. For example, rod-shaped *E. coli* and spherical *S. aureus* cells can be imprinted onto PDMS surfaces to create specific cavities. However, effective recognition also depends on surface chemical interactions, not just shape conformity. Therefore, the addition of functional groups such as phenylboronic acid (PBA) or the use of affinity polymers such as chitosan are often used to increase affinity to bacterial cell surfaces (Golabi et al., 2017).

For fungi such as *Saccharomyces cerevisiae*, studies have shown that surface imprinting of phospholipid-containing SIPs can enhance cell adhesion through long-range hydrophobic forces. The resulting cavities not only replicate the cell shape but also strengthen the interaction with the target cell membrane. Thus, MIPs/SIPs can be designed not only for single molecules but also for complex, multicomponent entities such as intact cells or virus particles. The successful identification of this biomarker is an important basis for the accuracy and sensitivity of electrochemical biosensors in the context of infectious disease diagnosis (Yongabi et al., 2018)

Clinical Applications of MIP/SIP Biosensors for Infectious Diseases

The use of MIP and SIP-based biosensors in clinical applications has shown great potential, particularly in the rapid and selective detection of pathogens that cause infectious diseases. The ability of MIP/SIP to be re-recognized by target analytes in complex environments such as blood serum, urine, or other body fluids is a key foundation for diagnostic applications. Unlike conventional detection techniques that require lengthy and

expensive sample processing, MIP biosensors enable direct analysis without special extraction or labeling steps. This makes them highly suitable for point-of-care (POC) diagnostic systems, especially in areas with limited facilities (Fauci & Morens, 2012).

Several clinical applications have been reported in the specific detection of bacteria. For example, *Staphylococcus epidermidis*, a Gram-positive bacterium that frequently causes nosocomial infections in catheters and implants, has been successfully recognized by SIP biosensors using a boronic acid-based surface imprinting technique. This polymer exhibits strong interactions with the sugar-containing bacterial cell wall, enabling detection at femtograms per milliliter in less than 10 minutes. Furthermore, *Proteus mirabilis*, a common cause of urinary tract infections, can be selectively detected with MIPs developed using a direct electropolymerization strategy on carbon electrodes (Shen et al., 2014).

The detection of *Escherichia coli*, a key indicator of microbial contamination in water and food, has also shown promising results using MIPs imprinted from intact cell walls. In a study by Gajdosová et al., MIPs were synthesized using soft lithography on a PDMS surface and successfully recognized *E. coli* specifically with a detection limit of 10^2 CFU/mL. Sensitivity was significantly increased by the addition of gold nanoparticles, which function as conductors and enhance electron transfer. This biosensor can also be reused more than 10 times without significant loss of performance (van Grinsven et al., 2016).

For virus detection, the use of virus-imprinted polymers (VIPs) offers a diagnostic approach that does not require biological reagents. One notable example is the detection of Zika virus using an electrochemical biosensor based on SIPs. In a study by (Wangchareansak et al., 2014), biologically inactive Zika virus was imprinted onto the surface of a graphene oxide-modified electrode. The resulting imprinting polymer was able to recognize the virus in serum samples with high sensitivity, and had a LOD of approximately 10^{-3} PFU/mL. This device demonstrated good selectivity against dengue

virus and other related viruses, suggesting potential for use in endemic areas [4].

Human papillomavirus (HPV), specifically HPV-16, which is closely associated with cervical cancer, has also been successfully detected using a MIP designed to recognize the oncogenic E7 protein. This MIP-based sensor exhibited high affinity even among homologous protein variations from other HPV types. Due to its stability, this sensor can be used for initial screening in high-risk populations. Furthermore, the sensor can be reused after simple rinsing without losing its binding ability to the target protein, thus reducing long-term costs (Cai et al., 2010).

In HIV infection, detecting the HIV-p24 protein, a key biomarker in the early phase of infection, is crucial for early diagnosis. Carbon nanotube- and 4-aminothiophenol-based microinjection (MIP) technology has been used to print the p24 protein, resulting in highly conductive cavities and an open 3D structure that supports rapid molecular diffusion. This biosensor achieved a detection limit of 10 pg/mL, sufficient to detect infection even before antibodies are formed in the patient's body. Thus, MIP provides a much-needed non-immunological diagnostic solution for detecting the window phase of infection (Ma et al., 2017). Going forward, MIP/SIP-based biosensors are predicted to become increasingly used in clinical diagnostics due to their numerous advantages, including stability, high sensitivity, and potential for reusability. The combination of nanotechnology and artificial intelligence paves the way for the design of intelligent biosensors that can not only detect biomarkers but also perform automated data analysis. Furthermore, integration with wearable devices and the Internet of Things (IoT) will enable real-time and distributed infection monitoring. To achieve this, challenges such as fabrication reproducibility, clinical standardization, and field validation trials still need to be addressed through further research and multidisciplinary collaboration.

Table 1. Performance of MIP/SIP biosensor

Target	MIP/SIP method	LOD	Linear Range	Ref.
AHLs	Fe ₃ O ₄ @SiO ₂ -MIP	10 ⁻¹⁰ M	2.5×10 ⁻⁹ –10 ⁻⁷ M	(H. Jiang et al., 2016)
Bacterial surface proteins	3-aminophenol electropolymer	0.60 nM	–	(Khan et al., 2016)
Bacterial flagella filaments	Phenol electropolymer	0.6 ng/mL	0.01–100 µg/mL	(Khan et al., 2017)
Staphylococcus epidermidis	3-APBA electropolymer	–	10 ³ –10 ⁷ CFU/mL	(Golabi et al., 2017)
Bacillus cereus spores	Pyrrrole electropolymer	10 ² CFU/mL	10 ² –10 ⁵ CFU/mL	(Ait Lahcen et al., 2018)
Zika virus	Prepolymer-GO composite (UV)	~10 ⁻³ PFU	10 ⁻³ –10 ² PFU/mL	(Altintas et al., 2015)

CONCLUSION AND SUGGESTION

Conclusion

Electrochemical biosensors based on molecularly imprinted polymers (MIPs) and surface-imprinted polymers (SIPs) have shown great potential as diagnostic platforms for infectious diseases. This technology addresses the need for rapid, sensitive, specific, and inexpensive detection methods that can be used directly in the field (point-of-care testing). Compared with biological receptors such as antibodies or enzymes, MIPs/SIPs offer significantly better chemical and physical stability and can be tailored to a variety of target analytes.

Theoretically, MIPs work by imprinting target molecules into a polymer matrix to form specific recognition cavities. The non-covalent interaction between the monomer and template allows for design flexibility and production efficiency. Electropolymerization processes and various surface-imprinting techniques have enabled the application of MIPs/SIPs directly to electrodes, supporting miniaturization and integration with electronic systems.

In practice, MIPs/SIPs can detect a wide range of infectious disease biomarkers, from small molecules such as bacterial metabolites

and signals, to toxic proteins, to complex entities such as viruses and microbial cells. Surface imprinting (SIP) approaches have proven effective for large targets, while volumetric imprinting techniques are suitable for small molecules. The use of appropriate functional monomers and reinforcement with nanomaterials further enhances sensor performance.

Numerous studies have demonstrated the effectiveness of MIP/SIP biosensors in detecting pathogens such as *E. coli*, *Staphylococcus epidermidis*, Zika virus, HIV, and toxins like aflatoxin B1 with high sensitivity and selectivity. This success demonstrates that MIPs/SIPs are not just conceptual technologies but are already reaching the stage of practical implementation for clinical applications.

Suggestion

To support the further development and widespread adoption of MIP/SIP biosensor technology in infectious disease diagnosis, several points need to be considered.

1. Standardization of sensor fabrication and characterization protocols is needed to ensure reproducible and clinically validated results. Variations in synthesis methods, electrode types, and polymer compositions still pose challenges to consistent sensor performance.
2. The development of MIP/SIPs for macromolecular and whole-cell targets needs to be further encouraged. Surface-printing approaches based on topography and surface chemical interactions require further exploration to maintain sensitivity even without labeling. This is crucial for direct pathogen detection in clinical samples without complex preparation.
3. Integration of MIP/SIP technology with portable and digital systems such as smartphone-based sensing, microfluidic chips, or IoT devices is key to addressing detection challenges in the field. Furthermore, the use of artificial intelligence (AI) in sensor signal analysis will improve diagnostic accuracy and

efficiency, particularly in multi-target data processing.

4. More clinical validation studies with large and diverse sample sizes are needed to ensure the sensor meets regulatory standards for widespread use in healthcare settings. Cross-disciplinary collaboration between materials scientists, molecular biologists, medical device engineers, and clinical practitioners is essential to accelerate the transition of this technology from the laboratory to real-world practice.

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