MIP Application for the Detection of Infectious Diseases

Subjects: Polymer Science

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Molecularly imprinted polymer (MIP)-based biosensors have enormous potential for disease detection. Infectious diseases can be detected and identified using MIPs, which are imprinted with whole viruses or specific proteins—biomarkers. Simple detection of the virus can be achieved by whole virus surface imprinting because viruses are easily identified by their morphology and surface properties. Other imprinting techniques and related sensitivity of the prepared MIP-based sensors are bulk imprinting, soft lithography, self-assembly, and the particle core-shell (template immobilization technique). Using MIP-based technology, viruses can be detected by a whole virus, as in the case of the Japanese encephalitis virus imprinted in the tetraethyl orthosilicate or hepatitis A virus imprinted in polydopamine (PDA), virus aptamer (e.g., HIV-1 gene imprinted in poly(o-phenylenediamine on ITO), main protein (e.g., spike protein or NS1 (non-structural protein 1—a specific and sensitive biomarker for dengue virus infection) or HIV-p24 (human immunodeficiency virus p24)), epitope (e.g., glycoprotein 41, gp41 (of related protein to human immunodeficiency virus type 1 (HIV-1))) templates.

molecularly imprinted polymer (MIP) electrochemical sensor infectious disease biomarker conducting polymer (CP) biomarker detection COVID-19 detection HIV-1 sensor Hepatitis C sensor sepsis detection inflammation detection SARS-CoV-2 sensor SARS-Cov-2

1. MIP Formation Principles

Electrochemical sensor design starts with the selection of particular electrode material. Pencil graphite electrode [1], graphite electrode [2], boron-doped nanocrystalline diamond [2][3][4], platinum electrode [5], gold of quartz crystal microbalance [6][7][8] or surface plasmon resonance sensor [9], etc. were used previously in the design of the electrochemical sensor. MIPs are formed from a solution containing functional monomers and an analyte, the so-called template molecule. Common imprinting techniques include bulk, particle, surface, and epitope imprinting (**Figure 1**). By adjusting electrochemical parameters, electropolymerization has unique advantages over other types of polymerization methods in controlling film thickness and porosity. This method is also fast, easy, and affordable. After polymerization occurs, the template molecules are extracted from the polymer matrix to leave complementary cavities. Previous studies have demonstrated MIPs for low molecular weight molecules [2][3][4][6][8]

[10][11] or large molecular weight objects (such as proteins [5][12], DNA [1], viruses , or bacteria [13]). The development of MIPs for large objects might be somewhat challenging [14] and extraction of the template molecule from the polymer matrix is considered to be one of the most complex processes.

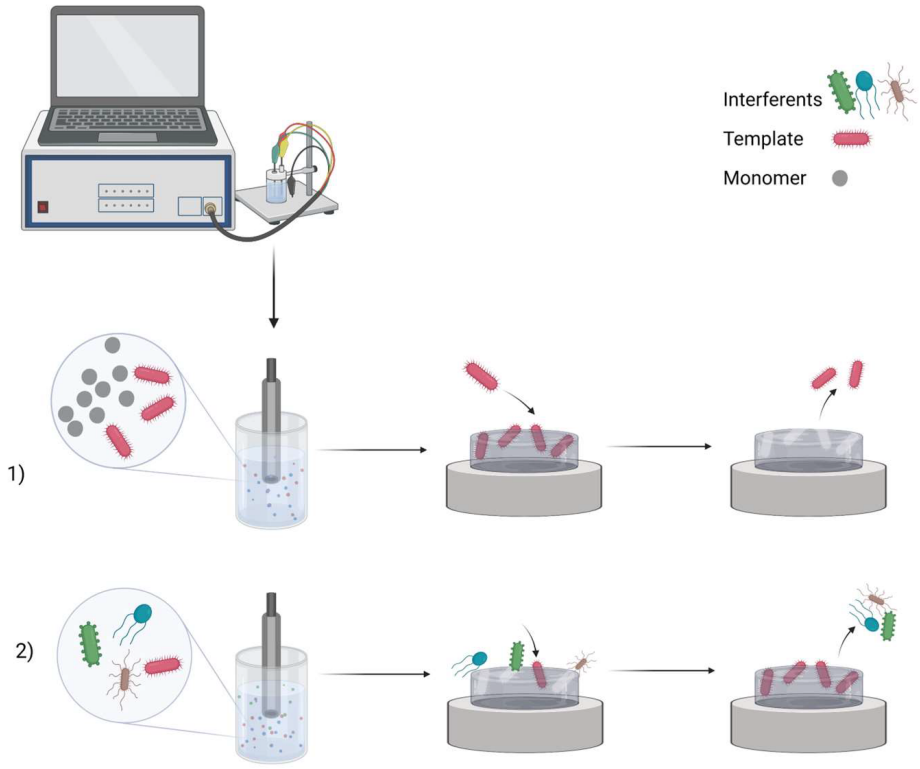

Figure 1. MIP preparation scheme: (1) imprinting template molecules and then washing them out; (2) rebinding process in the sample. Created with Biorender.com (accessed on 28 May 2023).

2. HIV-1

Lu et al. [15] developed a biosensor for the detection of protein gp41 related to HIV-1 based on imprinting of the epitope in PDA. Some advantages of epitope imprinting over whole protein imprinting include easier and cheaper template acquisition, more control in template conformation maintenance, easier template removal, and recognition site accessibility. The mixture of dopamine as a functional monomer and the synthetic peptide fragment 579–613 of gp41 as a template was coated on a quartz crystal microbalance chip. This QCM chip was able to bind the most templates when polymerized using 5 mg/mL of dopamine. Lower rebinding and subsequently lower sensitivity would result from a thicker film. The MIP was capable of recognizing not only the epitope template but also the entire gp41 macromolecule. The LOD of gp41 was reached at 2 ng/mL and was successfully used to monitor HIV-1 gp41 in human urine samples. This simple MIP method demonstrates how imprinted epitopes combined with CPs can be used for rapid biomolecular analysis.

Ma et al. [16] developed a sensitive electrochemical MIP biosensor constructed with conducting polymer Ppy for the detection of HIV surface protein gp120. Through an imine bond formed between glutaraldehyde's aldehyde groups and the amino groups of the protein, template molecules were covalently joined to the glassy carbon electrode

surface. As the free gp120 protein is conformationally unstable, its detection can be challenging. Combining free gp120 with NBD-556 (inhibitor of the interaction between gp120 and receptor) in the MIP biosensor improves recognition, linear range, and LOD of 0.0003 ng/mL by limiting the gp120 conformation.

Even though routine HIV testing of blood products has become standard, the increased transmission of transfusion-related diseases in underdeveloped nations is a result of the absence of quick, accurate, and affordable diagnostics. MIP-based electrochemical sensors, including whole protein or its epitope imprinting, together with CP, can be used to detect biomarkers of early HIV infection.

3. COVID-19

Ratautaite et al. [6] developed a sensor using molecular imprinting technology for the detection of SARS-CoV-2-S spike glycoprotein. Ppy was chosen as a CP film to entrap the template proteins. MIP (with imprinted template molecule cavities) and non-imprinted polymer (without imprinting) were synthesized on the Pt electrode. The results showed that the changes in MIP current are larger than in the non-imprinted polymer, and the sensor can be applied to the selective detection of imprinted SARS-CoV-2-S glycoprotein.

Ayankojo et al. [17] designed an electrochemical MIP sensor for a quantitative study of the SARS-CoV-2 spike protein. The SARS-CoV-2 spike protein was imprinted in a thin aminophenyl boronic acid polymer film using a surface imprinting technique. Only in the presence of fluoride ions can 3-aminophenyl boronic acid be electropolymerized into a CP. Real patients' nasopharyngeal samples were tested, and the LOD was 4.8 pg/mL. Furthermore, the proposed sensor was compatible with a portable potentiostat and can serve as a monitoring platform for COVID-19 patients for rapid and early diagnosis. The sensor demonstrated rapid diagnostic capability with a rebinding time of 15 min and a measurement duration of 5 min.

Also, electrochemical biosensing approach for detecting antibodies against SARS-CoV-2 spike proteins has been developed by immobilizing recombinant SARS-CoV-2 spike proteins on the surface of an AuE modified by a self-assembled monolayer. Cyclic voltammetry, differential pulse voltammetry, potential pulsed amperometry, and electrochemical impedance spectroscopy were chosen for the electrochemical evaluation [18].

According to the discussed articles, MIP-based electrochemical biosensors are a potential diagnostic strategy to be considered for biomarkers of SARS-CoV-2 infection.

4. Dengue Virus

A MIP-based impedimetric biosensor for dengue virus detection was developed by Arshad et al. [19]. It is a specific biomarker for dengue virus infection and was used as a template during the polymerization process to modify SPCE, which was subsequently coated with dopamine. To prepare the imprinting material, polysulfone nanofibers were made by electrospinning and later utilized as a support due to their substantial surface area and mechanical durability. NS1 concentrations as low as 0.3 ng/mL were selectively detected by the proposed sensor. According to

the authors of the study, the goal of achieving a specific and sensitive analysis succeeded because of the dopamine ability to self-polymerize at room temperature. This feature helped them retain the template's exact structure (NS1). In the final results, geometrically fit imprinted sites for specific detection of the target analyte were generated. Dengue virus has been detected in other studies using MIP technology with conducting [20] and non-conducting polymers. Buensuceso et al. [20] designed an MIP-based sensor on gold-coated QCM crystal for dengue NS1 detection using a terthiophene-based monomer (G03TCOOH) for epitope-imprinting. Terthiophene compounds have low oxidation potential, which makes them ideal for electrochemical functionalization and modification. A LOD of 0.056 µg/mL for NS1 protein was achieved, but the sensor was not tested in real-life samples. Overall, the sensor demonstrated long-term stability, high sensitivity, and selectivity.

In conclusion, both sensors showed satisfactory results for NS1 protein determination. However, the biosensor with PDA on the SPCE was more sensitive (LOD 0.3 ng/mL) than the epitope-imprinted sensor (LOD 0.056 µg/mL). This is probably due to the imprinting of the entire protein template and polysulfone nanofibers, which greatly increased the surface area. MIP-based biosensors can offer simple and cheap diagnostic devices instead of tourniquet tests that can be inaccurate or immunological-based tests that are time-consuming, laborious, expensive, and require complex equipment and highly qualified staff.

5. Hepatitis C Virus

Antipchik et al. [21] published the first report on the development of an MIP-based electrochemical sensor for detecting the hepatitis C virus via its surface protein E2. Green fluorescent protein was used in conjunction with E2 (total MW of 54 kDa) to prevent protein agglomeration and stabilize its structure. The MIP was prepared by electrochemical surface imprinting the E2 template molecule into poly(m-phenylenediamine) on the SPE electrode. As the proteins are large with unstable conformation, MIP imprinting can have some challenges, such as permanent template entrapment, lower mass transfer, denaturation, and diffusion, which limits the availability of imprinted cavities. The type of imprinted polymer—surface imprinted polymer (SIP) technique—can be advantageous to overcome these challenges. The ability to detect both free antigen E2 and the entire virus particle via E2 is a clear benefit of this technique. A LOD of 0.46 pg/mL and 15 min detection time indicate that the biosensor could be used for early-stage or chronic hepatitis C detection.

Ghanbari and Roushani [22] developed a novel biosensor for hepatitis C virus core antigen by electropolymerized dopamine around the aptamer (hepatitis C virus core antigen) complex on multi-walled carbon nanotubes-chitosan modified GCE. The improved properties of the MIP-aptamer dual recognition sensor include high sensitivity, low detection limit, high stability, and high selectivity. The results demonstrated that this biosensor could be used to detect HCV core antigens quantitatively in human serum. However, no selectivity studies have been reported. Chitosan is highly adhesive, water-permeable, membrane-forming, biocompatible, and prone to chemical modification due to its reactive hydroxyl and amino functional groups. The biosensor achieved a low LOD of 1.67 fg/mL and showed high stability.

In conclusion, the MIP-based sensor with E2 template poly(m-phenylenediamine) showed a sufficient LOD of 0.46 pg/mL, while the sensor that used the aptamer-antigen complex in the imprinting step had more sensitive results (LOD 1.67 fg/mL), likely due to the MWCN-Chi modification and aptamer affinity for the target protein. MIP-based electrochemical sensors have great potential for the diagnosis of hepatitis C infection.

6. Nosocomial Infections

Sharma et al. [23] fabricated a simple MIP-based electrochemical sensor for *K. pneumonia* bacteria (rod-shaped, 2 µm long, and 0.5 µm diameter) detection using a conducting polymer Ppy (a LOD of 1.352 CFU/mL). The oxidative polymerization reaction produced positively charged Ppy, which easily captured the negatively charged bacteria inside the polymer through weak electrostatic interactions; thus, the bacteria were easily removed from the polymer matrix by sonication and rinsed with deionized water. The electrochemical sensor was tested with five interferents: two other bacteria, *Lactobacillus* and *E. coli*, different ions (K^+, Mg^{++}) and molecules (urea, uric acid) that are present in human urine. As for testing interferences, no significant change was observed in DPV current peaks, though the peak was the lowest upon contact with *K. pneumonia* bacteria showing the highest affinity to the sensor. The sensor was also tested in urine samples. Later, Pintavirooj et al. [13] created a more sensitive MIP-based electrochemical biosensor consisting of three monomers to identify *K. pneumoniae*, resulting in a high linear response with a lower LOD of 0.012 CFU/mL. Methyl methacrylate (MMA), acrylamide (AAM), and N-vinylpyrrolidone (NVP) at a ratio of 2:1:1 gave the best linearity results. The specificity for the target *K. pneumonia* was the highest compared to the other two bacteria, *E. faecalis* and *P. aeruginosa*.

A simple MIP-based sensor consisting of a conducting Ppy layer on the ITO electrode showed efficient results for *K. pneumoniae* detection (LOD 1.352 CFU/mL) [23], but the more complex sensor consisting of three monomers mixture and graphene oxide combination on the gold SPE, it gives more sensitive detection (LOD 0.012 CFU/mL) [13].

Sarabaegi and Roushani [24] reported a sensor based on aptasensing and molecular imprinting for the detection of *P. aeruginosa* bacteria. GCE was covered with AuNPs, and then the aptamer-*P. aeruginosa* complex was immobilized on the electrode by PDA electropolymerization. The sensor showed excellent results in sensitivity and selectivity against *Shigella flexneri*, *Salmonella enteritidis*, *Escherichia coli*, and *Klebsiella pneumonia*. Results in real blood samples also showed a high (99–102%) recovery. With easy preparation, low cost, and high stability, this sensor can provide the detection of a variety of bacteria by imprinting linked aptamers, antibodies, or peptide fragments. Tokonami et al. [25] constructed an MIP-based sensor for the detection of *P. aeruginosa* using electropolymerized Ppy on the surface of Au-QCM. The characterization was obtained by di-electrophoresis. The overoxidation of Ppy allowed the formation of cavities that were shape-complementary to the template bacteria. Although the sensor had good selectivity, the LOD was significantly higher (10^3 CFU/mL) compared to the Sarabaegi and Roushani [24] reported sensor (LOD 1 CFU/mL).

Liustrovaite et al. [26] found that an SPCE electrode is more efficient than a Pt electrode for *L. monocytogenes* bacteria (0.5–2 µm-long) detection resulting in a LOD of 70 CFU/mL. Since template extraction is challenging for

MIP-based sensors, various extraction solutions (sulfuric acid, acetic acid, L-lysine, and trypsin) were tested. The team found that 10% acetic acid and proteolytic enzyme trypsin worked best to extract *L. monocytogenes* from the Ppy film.

A summary of the MIP application for the detection of biomarkers of infectious diseases is given in **Table 1**.

Table 1. Summary of the electrochemical sensors based on the molecularly imprinted polymers for the detection of infectious diseases HIV-1, COVID-19, Dengue virus, hepatitis C virus, and nosocomial infections biomarkers.

Biomarkers	Polymers and Modifiers	Electrodes	Extraction of Templates	Electrochemical Analysis Methods	LOD, LOQ, LR	Interfering Molecules	Reference
	HIV-1						
gp41	PDA	QCM	5% acetic acid (in H_2O) for five times, DI water	X-ray photoelectron spectrometer (XPS)	LOD 2 ng/mL; LR 5–200 ng/mL		[15]
gp120	Ppy, CNF-Bi, chitosan	GCE	Hyper pure water; methanol and acetic acid solution for 20 min.	CV, DPV	LOD 0.0003 ng/mL; LR 0.002–200 ng/mL	HIV-1 protein p24, human chorionic gonadotropin, carcinoembryonic antigen	[16]
	COVID-19						
SARS-CoV-2-S spike glycoprotein	Ppy	Pt	Incubation in 0.05 M H_2SO_4 for 10 min.	Pulsed Amperometric Detection		BSA	[6]
SARS-CoV-2 spike protein	Poly(aminophenylboronic acid)	SPE	50 mM dithiothreitol for 30 min; 30 min in 10% acetic acid	SWV, CV	LOD 1.12 pg/mL; LR 0–400 fM	SARS-CoV-2 nucleocapsid protein, E2, HSA, IgG	[17]
	Dengue virus						
NS1	PDA, polysulfone fibres	SPCE	PBS; 500 µg/mL of proteinase K for 2 h in the dark	EIS, CV	LOD 0.3 ng/mL; LR 1–200 ng/mL	FBS, lysozyme	[19]
NS1	Poly(G03TCOOH), gold	QCM	Potential washing (−0.7 V) 0.1 M	EIS	LOD 0.056	angiotensin II human, glycyl	[20]

Biomarkers	Polymers and Modifiers	Electrodes	Extraction of Templates	Electrochemical Analysis Methods	LOD, LOQ, LR	Interfering Molecules	Reference
			tetrabutylammonium hexafluorophosphate in acetonitrile		µg/mL; LR 0.2 to 10 µg/mL	glycine, bovine serum albumin, fibrinogen	
	Hepatitis C virus						
HCV surface protein E2	PmPD	SPE	PBS with 50 mM dithiothreitol for 30 min, 10% acetic acid solution on vortex for 30 min	DPV	LOD 0.46 pg/mL; LR 0.01–50 ng/mL; LOQ 15.3 × 10^{-5} ng/mL	HSA, IgG, CD81	[21]
HCV core antigen	PDA, MWCNTs- Chit nanocomposite	GCE	Water, overnight in 5% *v/v* acetic acid and 1% *w/v* cetyl trimethyl ammonium bromide in water with stirring	CV, DPV, EIS	LOD 1.67 fg/mL; LR 5.0 fg/mL to 1.0 pg/mL;		[22]
	Nosocomial infections						
K. pneumoniae	Ppy	ITO	DI water, ethanol	CV, DPV	LOD 1.352 CFU/mL; LR 1–105 CFU/mL	uric acid, K^+, Mg^{++}, urea, Lactobacillus, *E. coli*	[23]
K. pneumoniae	Poly(MAM:AAM:NVP), graphene oxide	AuSPE	10% acetic acid for 30 min, water at 50 °C for 30 min	CV	LOD 0.012 CFU/mL; LOQ 1.61 CFU/mL; LR 101–105 CFU/mL	*E. faecalis*, *P. aeruginosa*	[13]
P. aeruginosa	PDA, AuNPs	GCE	Solution containing SDS 0.01 M and 5% HNO_3 in water	CV, EIS, DPV	LOD 1 CFU/mL; LR 10–	*Shigella flexneri*, *Salmonella enteritidis*, *E. coli*, *K. pneumonia*	[24]

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diagnostic tests that identify specific pieces of SARS-CoV-2, HIV, dengue, etc. will open up the possibility of early detection of infectious COVID-19, HIV, dengue, sepsis, and other infectious diseases.

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Biomarkers	Polymers and Modifiers	Electrodes	Extraction of Templates	Electrochemical Analysis Methods	LOD, LOQ, LR	Interfering Molecules	Reference
					10^7 CFU/mL		
L. monocytogenes	Ppy	SPCE	10% acetic acid, or sulfuric acid, or L-lysin, or trypsin	PAD	LOD 70 CFU/mL, LOQ 210 CFU/mL, LR 300–6700 CFU/mL.		[26]

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PDA—polydopamine; D-Arg—polyarginine; BSA—bovine serum albumin; CD48—Cluster of Differentiation 48; HCV—Hepatitis C virus; FBS—fetal bovine serum; IgG—immunoglobulin G; PmRD—poly(m-phenylenediamine).

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