

## Recent advances in the application of molecularly imprinted polymers for electrochemical detection of biomedical markers

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### ABSTRACT

Late diagnosis of multifactorial diseases such as cancer, respiratory diseases, and infections continues to be a leading cause of high mortality rates, negatively impacts treatment outcomes, and leads to increased healthcare costs. Biomarkers that indicate the presence of various pathophysiological events, especially cancer, are critical for early diagnosis and monitoring of diseases. However, traditional analytical methods used for biomarker detection, such as chromatographic, spectroscopic, and electrophoretic techniques, have disadvantages such as long analysis times, the need for specialized personnel, and large reagent consumption. Electrochemical methods have emerged as promising alternatives that offer advantages such as faster analysis, cost-effectiveness, sensitivity, and reproducibility. Despite these advantages, electrochemical sensors face selectivity challenges. This problem is addressed by molecularly imprinted polymer (MIP)-based sensors designed to create selective recognition sites that match target molecules. With their high selectivity, specificity, cost-effectiveness, stability, and reusability, MIPs have been rapidly adopted for biomarker detection in various fields, including environmental, food, and biomedical applications. Miniaturized and portable MIP-based sensors further enhance the practicality of biomarker detection. This review covers the recent developments in MIP-based electrochemical sensors, focusing on their applications, sensitivities, and mechanisms for rapid and selective detection of key biomarkers. Additionally, it aims to provide insights and directions for future research in this field.

### KEYWORDS

Biomarker,  
electrochemical analysis,  
molecularly imprinted  
polymer, detection,  
sensor

### 1. INTRODUCTION

A set of illnesses known as cancer is characterized by aberrant cell proliferation that can infiltrate or spread to other bodily parts [1]. Biomarkers have an important place, especially in the evaluation of cancer cells. Biomedical parameters that indicate many deadly diseases, especially cancer, and various pathophysiological events in the body are called biomarkers. Detecting biomarkers has clinical

applications in many diseases affecting oncological, respiratory, and digestive tracts and systemic diseases [2,3]. Therefore, early, rapid, reliable, and sensitive detection of biomarkers in biological samples (blood, urine, etc.) is of vital importance.

In the literature, many methods have been used to detect biomarkers. These methods are chromatographic, spectroscopic, electrophoretic, etc., traditional analytical

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methods. However, although these methods have many advantages, they have many disadvantages, such as long analysis time, expert staff support, and the use of large organic solvents [2,3]. Electrochemical methods are widely used as an alternative method to overcome these disadvantages. Moreover, electrochemical methods are frequently preferred in sensor preparation due to their fast analysis time, reliability, cheapness, sensitivity, and repeatability [2]. However, electrochemical sensors still have deficiencies in selectivity. Therefore, the selectivity problem is overcome by integrating molecularly imprinted polymer sensors (MIP) [4].

The purpose of forming MIPs is to produce selective recognition cavities with a shape and functional group composition that directly corresponds to the target compound. The most suitable functional monomer for the target molecule is selected with the help of a cross-linker. In this way, the removal of the target molecule after the polymerization process ensures the formation of specific and selective cavities [2,3,5]. In addition to their excellent selectivity and specificity, MIPs offer stable, cheap, easy-to-prepare, and reusable possibilities [3, 6]. Due to the properties of MIP-based sensors, their areas of use in biomarker detection have grown rapidly, and there are many

application areas and rapid and selective detection opportunities in environmental, food, and biological samples [2,3]. Moreover, MIP-based sensors are important in detecting biomarkers when they can be integrated and transformed into miniature and portable devices. When the literature is examined, it is seen that MIP-based sensors are widely used to detect biomarkers, and there is a constantly growing interest in this area [1–3].

As a result, MIP-based electrochemical sensors have many applications in food, environmental, biomedical, and pharmaceutical fields and are widely used to detect biomarkers. Furthermore, the identification and detection of biomarkers are vital for human health. Therefore, this review will discuss the application areas, sensitivities, and mechanisms of MIP-based sensors developed in recent years for the rapid and sensitive detection of some important biomarkers. In addition, this study is intended to guide future studies.

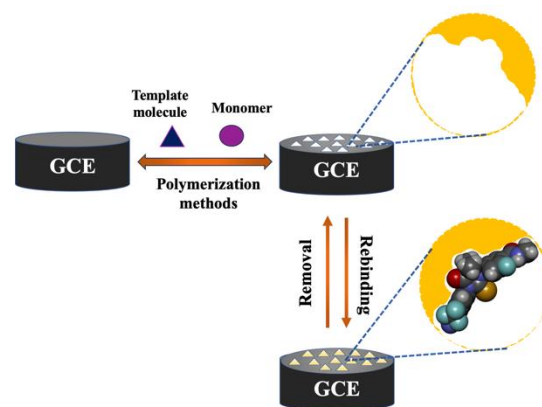
## **2. Molecularly imprinted polymers (MIPs)**

MIPs, distinguished by the particular recognition sites complementary to the size, shape, and functional groups of the template molecules [7], were first developed by Wulff and Klotz in the 1970s based on the MIT guiding principle [8]. In contrast to natural recognition elements

such as enzymes, antibodies, peptides, proteins, and so on, MIPs are considered artificially synthetic receptors that are superior to the natural ones in terms of physical robustness, chemical and thermal stability, specificity, synthetic cost, ease of storage, and reusability [9]. Therefore, MIPs have been widely used in drug delivery, immunoassay, separation and extraction, and sensing as significant alternatives for natural recognition elements [10–13]. Electrochemical analysis has several advantages over other analytical technologies, including quick reaction times, ease of use, affordability, and the potential for instrument miniaturization [14]. Developing electrochemical sensors with high stability, sensitivity, selectivity, and accuracy is crucial. However, transducers, sensing platforms, and recognition components may limit these capabilities. There has been a lot of interest in combining MIPs with electrochemical sensors since 1990 due to their unique host-guest identification process. The successful creation of MIP-based electrochemical sensors (MIP-ECSs) was made possible by advancements in polymerization techniques and detecting procedures. To create MIPs, a variety of polymerization techniques are used, namely sol-gel polymerization [15,16], bulk polymerization, suspension and

emulsion polymerization [17], and precipitation polymerization [18]. Furthermore, it can be separated into photopolymerization (PP), thermal polymerization (TP), electropolymerization (EP), and radiative polymerization (RP) based on the conditions in which the polymerization reaction is initiated. As illustrated in Figure 1, the preparation procedure is similar, even though the synthesis techniques and strategies for creating MIPs differ.

Functional monomers are usually needed to interact with the template (pre-assembly). The imprinted matrix is then created when monomers polymerize in the presence of templates with the help of an initiator and cross-linker. After producing complimentary cavities with templates, the templates can be extracted. The obtained imprinted sites can rebind and release the template or its structural analogs frequently since they possess selectivity towards them.



**Figure 1.** Schematics of the general preparation process of MIPs

## 3. Biomarkers

- **Lactate (LAC)**

Lactate (LAC), which has a complex and multifaceted structure in the human body, is tightly regulated to maintain the overall metabolic structure [19]. In addition, LAC is an important metabolite that changes during sweating in humans and is generally limited to difficult and intense activities. Therefore, irregular and unbalanced LAC production can indicate abnormal conditions such as respiratory failure, hypoxia, and lactic acidosis [20]. Moreover, LAC concentration is a critical measurement widely used in clinical diagnostics to investigate the health of patients and the diseases it causes [19, 20].

- **Bovine Serum Albumin (BSA)**

Albumin, which plays an important role in maintaining nutritional irregularity, plasma pressure, and the transport of different structures, is of vital importance to human health. Bovine serum albumin (BSA) is a protein found in large amounts in the blood and has a high structural similarity to human serum albumin (HSA). For this reason, BSA has been used as a model protein instead of HSA in different areas. BSA is transmitted to humans through foods obtained from cattle. When there is an excessive intake of BSA, some serious diseases (mad cow disease, insulin-dependent diabetes mellitus, etc.) occur. For this reason, studies are increasing in

many areas for the detection of BSA in biological and food [21, 22]

- **Prostate-specific Antigen (PSA)**

One of the most common types of cancer in men is prostate cancer. This type of cancer is the second most common cancer that results in death in older men. Therefore, the most important step in diagnosing this cancer is the detection of the prostate-specific antigen (PSA) protein in the blood. PSA is a protein secreted from the prostate gland and passed into the blood [23]. Therefore, PSA is a very important biomarker in the early diagnosis and prognosis of prostate cancer in the field of oncology [24]

- **Sarcosine (SAR)**

Prostate cancer occurs in the prostate tissue of a malignant tumor in men and is a cancer with a high mortality rate in men. Another biomarker widely used in the diagnosis of prostate cancer is sarcosine (SAR) [25]. SAR levels used to diagnose prostate cancer are detected in urine, tissues, or blood with increasing levels. Therefore, rapid and sensitive detection of SAR biomarkers is of great importance for human health [26]

- **Cardiac Troponin I (cTnI) and T (cTnT)**

Cardiac troponins are regulators of skeletal and cardiac muscle contractions. These troponins consist of cardiac troponin I (cTnI) and cardiac troponin T (cTnT) [27].

cTnI, produced only in the heart muscle, is a biomarker used to detect acute microcardial infarction (AMI). cTnT is another biomarker used to diagnose AMI. Moreover, this biomarker is used as a cardiac biomarker and as a guide in early diagnosis and patient follow-up. AMI is known to be one of the factors that result in the most deaths in the world. Therefore, rapid, sensitive, and reliable detection of cTnI biomarkers is very important for the early diagnosis of AMI [28–30].

- **Carcinoembryonic Antigen (CEA)**

Carcinoembryonic antigen (CEA) is a widely used marker for tumor detection. CEA is a glycoprotein found in excess in malignant tumors, especially in the diagnosis of diseases such as colon, stomach, lung cancer, and breast carcinoma. The CEA level in the blood must be determined for tumor progression. Therefore, biological determination of CEA level is very important for early diagnosis and treatment of tumors [31–33]

- **Amyloid- $\beta$  Oligomer (A $\beta$ O)**

Amyloid- $\beta$  oligomers (A $\beta$ O) are an important molecular biomarker that plays an important role in Alzheimer's disease (AD). It causes progressive and fatal outcomes for humans, primarily due to the rapid aging of the population. Therefore, reliable and rapid analysis of A $\beta$ O is of

vital importance in the early diagnosis and prognosis of AD [34, 35]

#### 4. Application of molecularly imprinted polymers for electrochemical detection of biomedical markers

When the studies in the literature are evaluated, it is clear that there is a recent trend toward identifying biomarkers, and numerous studies have been conducted in this field. Table 1 summarizes the recent electrochemical sensor applications to analyze various biomarkers in terms of basic sensor properties, including technique, linear range, LOD, applications, and recovery percentage.

**Table 1.** MIP-based electrochemical sensors for analysis of various biomarkers

Biomarker	Sensor	Method	Linearity range	LOD	Application	Recovery(%)	References
PSA	GS-AuNP/CS/GCE	DPV	1 pg mL <sup>-1</sup> -100 ng mL <sup>-1</sup>	0.15 pg mL <sup>-1</sup>	Women serum sample	92.00-103.50	[36]
PSA	Ab-GO-MWCNTs-Fe <sub>3</sub> O <sub>4</sub> /SPCE	EIS	0.01-100 ng mL <sup>-1</sup>	5.4 pg mL <sup>-1</sup>	Serum Urine	98.17-98.73	[37]
PSA	SPCE/MIP/PE DOT:PSS@Fc	DPV	1.0×10 <sup>-7</sup> -1.0 × 10 <sup>-4</sup> ng mL <sup>-1</sup>	8.3 ± 1.3×10 <sup>-8</sup> ng mL <sup>-1</sup>	Human serum sample	89.4 ± 7.4-102.9 ± 6.4	[38]
LAC	3-APBA/Ag-NWs/SPCE/MIP	DPV	10 <sup>-6</sup> - 0.1 M	0.22 μM	NR	NR	[39]
LAC	4-ABA/LAC/ZIF-8@ZnQ@MIP-GCE	DPV CV EIS	0.1-1.0 pM	29.9 fM	Human serum sample	99.23- 100.38	[19]
LAC	o-PD/AuNPs/RGO/GCE	DPV	0.1-1.0 nM	0.09 nM	Sugarcane vinasse sample	99.70-104.80	[40]
BSA	MIP/Pyr/chit/GCE	DEIS	0.0001-0.01 ng mL <sup>-1</sup>	5×10 <sup>-5</sup> ng mL <sup>-1</sup>	Human serum sample	99.40-102.00	[41]
BSA	ACR/DIA/BisAM/GCE	EIS	1.5×10 <sup>-16</sup> -10 <sup>-12</sup> M	7.2×10 <sup>-18</sup>	Serum sample	97.80-103.60	[42]
CEA	FTO/PPy-MO DMIP	CV EIS	5×10 <sup>4</sup> -10×10 <sup>4</sup> pg mL <sup>-1</sup>	1.6-3.3 pg mL <sup>-1</sup>	Human serum sample	96.00-98.80	[43]
SAR	MAA/CPE	DPV	5.0 μM-1.1 mM	0.38 μM	Urine sample	97.4-103.8	[44]
SAR	Fe <sub>3</sub> O <sub>4</sub> @ZIF-8@MIP	CV	1-100 pM	0.4 pM	Urine sample	96.8-105.8	[45]
cTnT	PANI/PMB/f-MWCNTs/SPCE	DPV	0.1-8.0 pg mL <sup>-1</sup>	0.04 pg mL <sup>-1</sup>	Human plasma sample	91.00-112.00	[46]
cTnI	MB/ZnONPs/GCE	DPV	0.50-3.3 × 10 <sup>5</sup>	1.04 pM	Human serum sample	93.40-114.28	[47]
cTnI	PPy/BNQDs/GCE	DPV	0.01-5.0 ng mL <sup>-1</sup>	0.0005 ng mL <sup>-1</sup>	Plasma samples	97.73-101.08	[48]
AβOs	Chit/ SiO <sub>2</sub> @Ag NPs/GCE	DPV	0.5-10 ng mL <sup>-1</sup>	1.22 pgmL <sup>-1</sup>	Human serum samples	93.00- 107.70	[49]

NR: Not reported, PSA: Prostate-specific antigen, LAC: Lactate, BSA: Bovine serum albumin, CEA: Carcinoembryonic antigen, SAR: Sarcosine, cTnT: Cardiac troponin T, cTnI: Cardiac Troponin I, AβOs: Amyloid-β oligomer, GS: Graphene sheets, AuNPs: Gold nanoparticles, CS: Chitosan, Ab: Antibody, GO: graphene oxide, MWCNTs: multi-walled carbon nanotube, PEDOT: poly(3,4-ethylenedioxythiophene), Fc: Ferrocene, 3-APBA: 3-aminophenylboronic acid, AgNWs: Ag Nanowires, 4-ABA: 4 aminobenzoic acid, ZIF-8: Zeolitic imidazolate framework-8, ZnQ: Zinc oxinate, o-PD: o-phenylenediamine, RGO: Reduced graphene oxide, Pyr: Pyrrole, Chit: Chitosan, ACR: Acrylic acid, DIA: Dialylamine, BisAM: N,N-methylenbisacrylamide, PPy: Polymerized polypyrrole, MO: Methyl orange, DMIP: Dual-template molecularly imprinted polymer, MAA: Methacrylic acid, PANI: Polyaniline, PMB: Polymethylene blue, MB: Methylene blue, ZnONPs: Zinc nanoparticles, BNQDs: Boron nitride quantum dots, SiO<sub>2</sub>@AgNPs: Silver nanoparticles with silica

nanoparticles, FTO: Fluorine-doped tin oxide electrode, SPCE: Screen-printed carbon electrode, GCE: Glassy carbon electrode, CPE: Carbon paste electrode, EIS: Electrochemical impedance spectroscopy, DPV: Differential pulse voltammetry, CV: Cyclic voltammetry, DEIS: Dynamic electrochemical impedance spectroscopy K. Phonklam et al. [50] introduced a novel

electrochemical sensor based on cardiac troponin T (cTnT), an important biomarker for early diagnosing and treating acute myocardial infarction. The sensor used a molecularly imprinted polymer (MIP) technique combined with a screen-printed carbon electrode (SPCE) modified with multi-walled carbon nanotubes (MWCNTs) and an electro-coated polymethylene blue (PMB) redox probe. The sensor sensed the changes in PMB current according to cTnT concentration by differential pulse voltammetry. The sensor showed excellent performance with a linear detection range of 0.10 to 8.0 pg/mL<sup>-1</sup>, a detection limit of 0.040 pg/mL<sup>-1</sup>, and high sensitivity, selectivity, and binding affinity. It retained more than 90% of its sensitivity after 6 weeks of storage at room temperature. When applied to human plasma, the sensor results were consistent with the gold standard electrochemiluminescence method. This MIP-based electrochemical sensor shows significant potential for point-of-care (POC) testing applications.

In another study, Zhang et al. [51] advanced a wearable electrochemical biosensor based on silver nanowires (AgNWs) and molecularly imprinted polymers (MIPs) to noninvasively monitor

lactate in human sweat. The biosensor constructed using 3-aminophenyl boronic acid (3-APBA) by electropolymerization showed strong sensitivity and specificity in detecting lactate concentrations ranging from  $1 \times 10^{-6}$  M to 0.1 M, with a detection limit of 0.22  $\mu$ M. The sensor maintained high stability, maintaining  $99.8 \pm 1.7\%$  of its sensitivity even after 7 months of storage. Moreover, it showed good flexibility, maintaining reliable performance even after being bent and turned 200 times. When used on human skin, the sensor successfully monitored lactate levels in sweat, demonstrating its potential for application in sports, military, and healthcare.

Finally, Khumngern et al. [52] developed an MIP-based electrochemical sensor to detect prostate-specific antigen (PSA). MIP made of o-phenylenediamine (o-PD) and o-aminophenol (o-AP) was electropolymerized in the presence of PSA and deposited onto a screen-printed carbon electrode (SPCE) modified with a poly(3,4 ethylenedioxythiophene):poly(styrenesulfonate) composite with ferrocene (PEDOT: PSS@Fc). This modification increased the electrode conductivity and aided in the retention of ferrocene, increasing the peak current during PSA detection. The sensor showed a linear response over the PSA



concentration range of  $1.0 \times 10^{-7}$  to  $1.0 \times 10^{-4}$  ng/mL and had a detection limit of  $(8.3 \pm 1.3) \times 10^{-8}$  ng/mL. Over seven weeks, the sensor demonstrated good selectivity, reproducibility, reusability, and long-term stability. Langmuir adsorption fit showed a high affinity for PSA with a dissociation constant (KD) of  $1.91 \times 10^{-6}$  ng/mL. When tested in blood serum samples, the results were consistent with the electrochemiluminescence immunoassay method ( $P > 0.05$ ), confirming the reliability of the sensor.

## 5. Conclusions

In recent years, most of the deaths due to multifactorial diseases are due to late diagnosis and high medical care costs. Therefore, selective, reliable, sensitive, and rapid analysis of biomarkers for treating these diseases is of vital importance and is needed. In order to meet this need, many MIP-based electrochemical sensors have been developed by taking advantage of the physical and chemical durability of MIPs and, most importantly, their selective properties. The superior performance of MIP-based electrochemical sensors enables them to be integrated and implemented in miniature devices at an affordable cost to detect biomarkers. Moreover, these sensors are used to detect medical diagnoses without harming human health, without requiring expert knowledge, and in a

simple manner, providing significant advantages in the commercial field. The developed MIP-based electrochemical sensors provide reusable, durable, stable, reliable, rapid, and selective results in analyses in both biological and pharmaceutical fields and are expected to have an important place in many future applications.

## 6. Ethics approval and consent to participate

This study does not need any Ethics report.

## 7. Consent for publication

The Authors give consent for publication.

## 8. Availability of data and materials

All data and materials of the paper are available to the public.

## 9. Authors' contributions

Fatma BUDAK: Writing – original draft, Investigation, Conceptualization. Ensar PISKIN: Writing – original draft, Investigation, Conceptualization. Ahmet CETINKAYA: Writing – original draft, Investigation, Conceptualization. Sibel A. OZKAN: Writing – review & editing, Supervision.

## Disclosure statement

No potential conflict of interest was reported by the author(s).



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