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Sensor-based MIP technologies for targeted metabolomics analysis

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ABSTRACT

In recent years, metabolomics, identification and profiling of metabolites, have gained broad interest compared to other omics technologies and are progressively being utilized for biomarker discoveries. Therefore, the application of metabolomics in different fields are increasing day by day because of its high throughput results. However, the application of metabolomics requires state-of-the-art analytical approaches for the analysis. The complexity and limited availability of these instruments are restricting parameters for applying metabolomics studies in routine analysis. This problem may be overcome with molecularly imprinted polymer (MIP)-based electro sensors since they have high selectivity, sensitivity, easy applicability, portability, and low cost. This is the final step before developing end point-of-care tests (POCT), which patients can easily apply. MIP sensors will have more applications in the targeted metabolomics analysis to develop POCT systems. This review analyzes various metabolites using MIP-based electrochemical methods for their potential usage as POCT and biomarker research based on targeted metabolomics analysis requirements. The future applications for the sensitive assay of metabolites in medicine and clinical trials are also discussed.

1. Introduction

Metabolites are critical biological compounds that take part in physiological processes and tasks maintained in all living cells. The identification and profiling of metabolites can provide insight into the physiological condition of an organism [1]. However, the complexity, diversity of metabolic pathways and huge number of metabolites complicate identification, analysis, and evaluation of normal/pathological processes. One of the most up-to-date approaches to understanding, interpreting, and using this complex system for various purposes is metabolomics [2,3]. In this context, metabolomics is a novel approach that uses analytical chemistry to quantitatively identify, profile, and evaluate metabolites and metabolic pathways [4,5]. Therefore, metabolomics, which is also useful for genotyping, has excellent potential to be applied in medical fields for diagnosis and/or monitoring of treatment processes [6].

The most commonly used samples in analyzes are biological specimens such as serum, urine, tissues, cerebrospinal fluid, and saliva samples collected from human subjects. Although these samples are informative in many aspects, such as diagnosis and treatment of diseases, their analysis is quite challenging because they are complex matrixes containing thousands of metabolites, proteins, and other xenobiotics together [7]. Overcoming this challenge is the main focus of researchers by developing rapid, selective, sensitive, and affordable analytical sensors. Numerous studies are available to determine metabolites with spectrometric and chromatographic techniques, which are expensive, time-consuming, and complex methods [2,8]. On the contrary, electrochemical methods provide highly sensitive, fast, simple, affordable, and green analysis for metabolites [9].

Nevertheless, the most critical disadvantage of electrochemistry is low selectivity. Molecularly imprinted polymers (MIPs) can be defined as tailor-made synthetic mimic materials made with functional and cross-linking monomers (if necessary) in the presence of the target molecule. Since MIPs provide excellent selectivity with their analyte-specific nature, MIPs-based electrochemical sensors offer an enhanced platform combining the advantages of MIP technologies and electrochemistry [10,11]. These advantages allow the frequent use of MIP in point-of-care test (POCT) devices that patients and healthcare professionals can easily apply. It is

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considered that MIPs-based electrochemical sensors are a good starting point for POCTs that serve the future of targeted metabolomics analysis.

To the best of our knowledge, there are currently no published reviews on the analysis of small metabolites using MIP technologies based on electrochemical sensors. In this review, analysis of various metabolites using MIP-based electrochemical methods are evaluated for their potential usage as POCT and biomarker research based on targeted metabolomics analysis requirements. Moreover, the selection of templates and applications based on the sample types is also reviewed. This review provides a more focused and elaborate perspective on the analysis from untargeted metabolomics assay to MIP-based targeted metabolomics analysis. Furthermore, a well-known topic, MIP-based electrochemical sensors, is explained in a novel and significant perspective by overviewing the most recent studies in the literature. This review will also be helpful for the researchers planning to study in this area by offering a fundamental approach and discussing of the advantages and disadvantages of the available studies.

2. Metabolomics

The basic omics disciplines, which are genomics, transcriptomics, proteomics, and metabolomics, cover almost all biological and biotechnological research [12,13] (Fig. 1). Metabolomics is the identification and quantification of metabolites (molecular weight <3000 Da) found in cells, tissues, organs, and biological fluids over a certain time period [2]. A wide variety of metabolites can be detected with metabolomic analyzes, and the altered metabolites can be identified with chemometric methods. These metabolites enable us to understand cellular metabolism and shed new light on disease diagnosis, treatment response analysis, and explanation of underlying causes [14].

Phenotypes can vary according to epigenetic differences and the physiological or pathological state of the living organisms. Changes in the number of metabolites are affected by genetic and environmental factors as well as age, drugs, diseases, toxins, microbiota, nutrition, and lifestyle [12]. Therefore metabolomics is the best omic technique to represent phenotypes [2]. In metabolomic studies, comprehensive and simultaneous analysis of many metabolites from different chemical groups is performed to create metabolomic profiles [15]. This allows defining the phenotype and identifying specifically differentiated biomarkers due to a specific disease, treatment, lifestyle, microbiota, etc.

In general, the metabolomics workflow consists of sample preparation, analysis of metabolites, and data analysis, as shown in Fig. 2. The sample preparation step is critical because sample collection and storage conditions directly affect metabolite levels and the metabolomic profile. The method to be used in the metabolomics analysis must be selected according to the aim of the study. Metabolomic analysis can generally be performed with two main analytical approaches as targeted and untargeted metabolomic analyses. Untargeted assays focus on discovering new biomarkers, while targeted assay aims to quantify known metabolites with high accuracy and selectivity. Today, different analytical platforms are being used for metabolomic analysis to find more disease-specific metabolites and achieve high reproducibility [16].

Nuclear magnetic resonance (NMR) and mass spectroscopy (MS) combined with liquid (LC), or gas chromatography (GC) are the main techniques used in metabolic studies. NMR is a highly reproducible technique that identifies chemical structures, but LC-MS and GC-MS techniques are widely used due to their higher sensitivity and selectivity. In addition, electrochemical methods such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), and adsorptive stripping voltammetric methods, etc., are preferred in targeted metabolite analyses with high sensitivity, fast results, low cost, and miniaturization [17].

Quantitative/semiquantitative metabolites levels, determined in data analysis, are performed to create a data matrix. This big data can be analyzed with multivariate statistical analysis to reflect changes in the metabolomic profiles. Bioinformatics interfaces such as the Kyoto Encyclopedia of Genes and Genomes (KEGG), Max Library Database, Metabolite and Chemical Entity database (METLIN), Human Metabolome Database (HMDB) are used to obtain meaningful outputs from metabolomics [18,19].

In recent years, metabolomics has gained a wide of interest compared to other omics technologies and is progressively utilized for biomarker discoveries. However, the application of metabolomics analysis requires state art of technology instruments. The

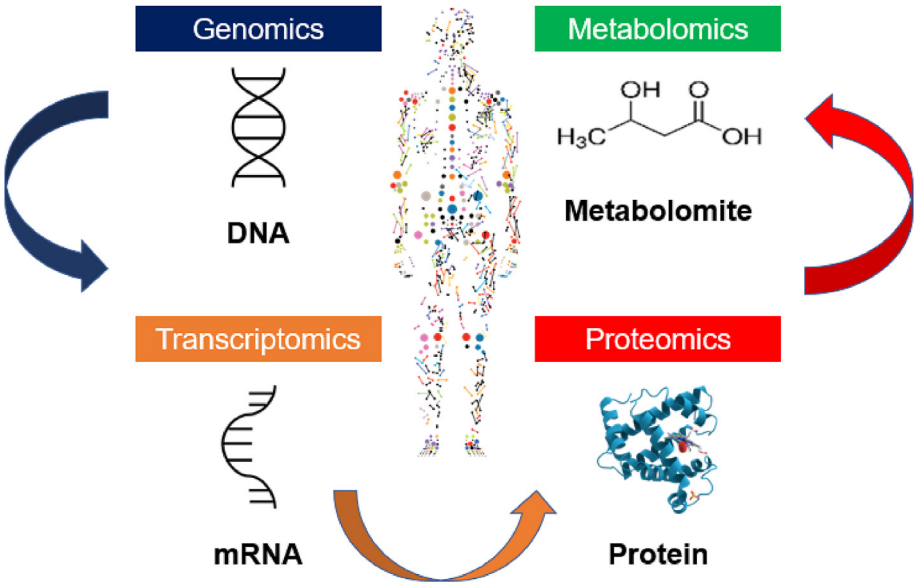

Fig. 1. The omics cascade in systems biology approach: Genomics, transcriptomics, proteomics, and metabolomics.

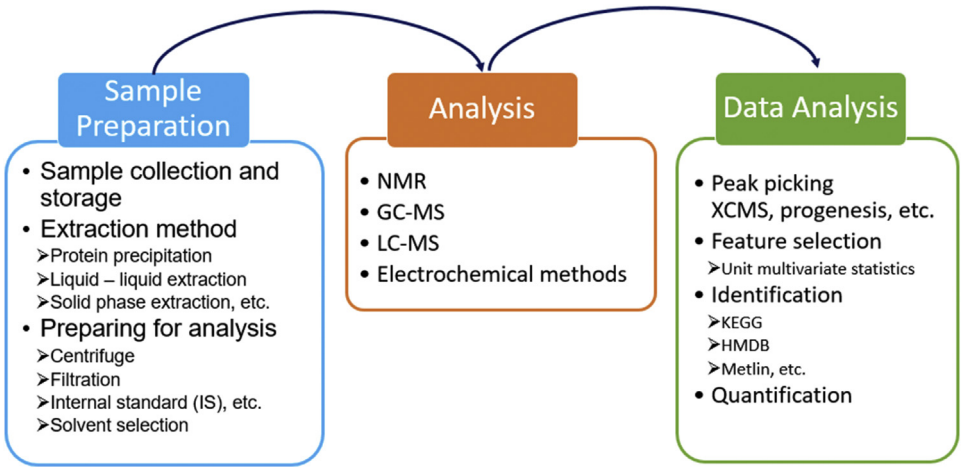

Fig. 2. Metabolomics workflow: Sample preparation, analysis, and data analysis.

complexity and limited availability of these instruments are restricting the application of metabolomics studies for routine analysis widely. This problem may be overcome with MIP-based electrosensors, since they have high selectivity, sensitivity, easy applicability, portability, and low cost.

3. Imprinted technology for targeted metabolomics analysis

Dickey was the first scientist to mention the molecularly imprinted principle in 1949. The principle was based on key-lock interaction. Target molecules (templates) and monomers identify each other, and the interaction between them can be non-covalent. The interaction between template and monomer can be non-covalent [20].

The polymeric matrix is referred to as an artificial antibody and mimics the biological receptor. It is a tailor-made high selective/sensitive receptor (recognition side) to recognize the target molecule. Moreover, the system can be mass-produced and easily miniaturized in a short period. Therefore, it can be used for the quick read-out of the target on-site without any professional supervision. In other words, the system can be used as point-of-care (POC) devices with excellent performance because of their user-friendly, rapid assessment, and portability [21]. Therefore, molecularly imprinted systems have a critical role because of their well-sensing tools in health care.

Molecularly imprinted strategies are based on five main imprinting approaches: bulk, particle-based, surface, micro-contact, and epitope imprinting. Most of the macromolecular imprinting approaches belong to surface and epitope imprinting in sensor applications. For the small molecule, the bulk imprinting approaches are widely used for the development of MIP sensors. The surface of the transducer is coated with a mixture of monomers and a small molecule template. The advantage of this type of imprinting is most convenient to obtain binding cavities to relatively small molecules because of the creation of binding sites within the polymeric bulk [22].

The bulk imprinting is based on the synthesis of 3D imprints, which have binding sites in the polymeric matrix. The typical bulk imprinting strategies are hardly applied for the macromolecular structure of imprinting to the polymeric matrix. Their reduced mass transfer, the non-homogenetic binding site, solvent selection, permanent entrapment in the polymeric matrix, and poor interaction with polymer and target can be given as a reason for the limitation of typical bulk imprinting strategies [20].

MIPs synthesized using the surface imprinting technique are approximately 20 times faster than the bulk imprinting technique. Moreover, surface imprinting techniques allow the production of controlled thin layers with favorable binding kinetics.

An epitope is referred to as a short amino acid sequence that is recognized by an antibody. For synthesizing protein imprinted polymer, epitope imprinting technology is widely utilized in the presence of conventional monomers and cross-linkers. In this imprinting method, the template is easily removed from the polymeric matrix [23]. Furthermore, the imprinting of whole protein is tricky due to its fragile and large structure, and the cavities of protein cause the non-specific binding. Therefore, specific epitope imprinting is preferred as a template compared to the whole protein imprinting for developing the MIP-based sensor [24]. For this reason, epitope imprinting can be shown as a perfect candidate for the determination of the small active site of larger biomolecules.

Proteins have an important role as they help in the early detection of some diseases. Biomimetic receptors are produced explicitly for the recognition of proteins. Metabolomics is the study of the measurable changes in the endogenous and exogenous metabolite profiles in biological systems. Metabolomics is widely used to discover vital biomarkers in clinical analysis. Several metabolites, purines, lipids, amino acids, pyrimidines, carbohydrates, peptides, and vitamins, etc., are covered in the metabolome. These biomolecules contribute to biosynthesis and biodegradation pathways. The metabolome can be known as low molecular weight (typically <1000 Da) biomolecules. The whole molecules or specific epitopes can imprint onto polymeric matrix via electrostatic interactions, non-covalent and covalent bonds for designing the binding sites for these target molecules. The computational modeling is performed previous to the synthesis of the polymeric matrix. Then, the results can give a valuable opinion for the selection of monomers and the interaction between monomers and templates.

3.1. MIP-based sensors

Biorecognition elements are the natural receptors (Antibody, DNA probes), while artificial receptors are alternatives for biosensing. These receptors have many advantages compared to natural receptors. The surface can be regenerated with the washing procedure and used for multiple analyses. The monomers used for MIP are durable to thermal and chemical changes and are not easily affected by environmental factors [24]. Unlike antibodies, MIPs can

be stored for a long time under optimum ambient conditions without any reduction inactivation.

Moreover, MIP contributes to the analytical separations/extraction and its determination, chemical sensors, purification, and enzyme-like catalysis. On the other hand, MIP-based sensors need to overcome some obstruction. For example, during the removal process, the templates may not be totally removed from the polymeric matrix, and therefore, the target has difficulty rebound the accessible cavities.

The published papers were extensively searched to report MIP-based electrochemical sensors of a wide range of targets. The development of MIP-based electrochemical sensors is an extremely popular, ever-growing, and diverse area of research, with approximately 1000 research papers published in the last five years. This data belongs to one-third of all published related articles (Fig. 3).

MIP-based sensors are fabricated in the presence of biorecognition elements and target systems. Biorecognition elements mimic the biomolecule receptor by polymeric matrix. The polymeric matrix is made by functional monomer, cross-linker, initiator, and target molecule in the presence of suitable media. The synthesized polymeric matrix provides stability, sensitivity, selectivity, and robustness to MIPs. This leads to the widespread use of MIPs in biosensing, including optical, electrochemical, and photoelectrochemical techniques [25].

MIP film thickness has a crucial role in a polymeric matrix. MIP film can be arranged with different polymerization approaches such as electrochemical deposition of conductive and non-conductive monomers, self-assembled monolayer (SAM), and controlled radical polymerization [26]. Electropolymerization techniques are widely applied to the electrode surface utilizing an interaction of MIPs and electrochemical detection for obtaining sufficient MIP layer thickness in the presence of a wide range of target biomolecules. The methodology is based on polymers formation through a potential application, causing oxidation or reduction in the monomers [20].

MIP-integrated systems are widely known as chromatographic systems, lab-on-a-chip systems, and sensor systems [27]. Moreover, MIP integrated devices are fabricated for point-of-care (POC) sensors. Thus, it contributes to enhancing the selectivity of a target biomolecule, rapid assessment, low-cost analysis, and offering an alternative to traditional methods in biosensing applications [28].

A brief description of the fabrication of MIP-based electrochemical sensors is that transducer is electropolymerized with polymeric matrix in the presence of template and monomer. After electropolymerization, the transducer is exposed to the removal solution to create cavities' specific size, shape, and functionalities. After the removal process, the target is effectively trapped in cavities in the polymeric matrix. Then, the non-imprinted polymer (NIP) sensors are fabricated to control the MIP sensors without the template in the same way. The imprinting factors (related to the ratio of MIP and NIP) prove the effectiveness of MIP-based sensor performance.

3.1.1. Template

The templates applied in recent years are found in a wide range from low-molecular-weight compounds (<1500 Da) to macromolecules(e.g., proteins) (>1500 Da). Metabolomics, a novel "omics", uses methods based on low-molecular-weight molecules, with the high-throughput evaluation of a large number of metabolites that may lead to the identification of new disease-specific biomarkers and the elucidation of pathophysiological mechanisms. Moreover, enzymes and peptides derived from disease-specific proteins are given as examples of low-molecular-weight compounds. Thus, MIP-based sensors can determine from inorganic ions to drugs, nucleic acids, and proteins.

3.1.2. Functional monomer

MIPs are synthesized with polymerization in the presence of templates to obtain unique cavities for a target molecule. The functional monomers encompass around the target molecules; consequently, a 3D polymeric structure is obtained. The ratio of functional monomer and template plays a critical role in providing successful target molecule imprinting. The polymeric matrix should save its stable thermodynamic condition in the imprinting process. On the other hand, the template should be easily removed from the polymeric matrix in the template removal process. The other parameter is the solution of the polymeric matrix, and it affects the affinity of the synthetic bioreceptor to a template

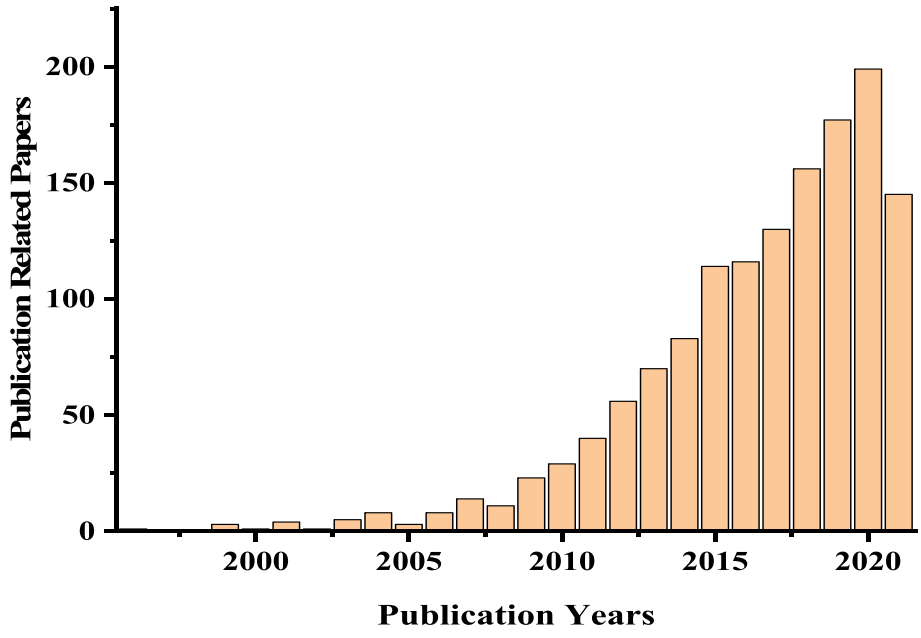

Fig. 3. Number of published papers related to MIP-metabolomics sensors according to publication years (collected from Scopus, September 20, 2021).

molecule. If cross-linker and initiator are needed, all the parameters should be optimized.

Non-conductive and conductive polymers are utilized for the synthesis of MIP-based sensors. Conductive polymers are organic compounds and show high optical and electrical multifunctional properties. Thus, they contribute to enhancing to immobilization of bioreceptors in optical and electrochemical biosensing. Moreover, the synthesis of conductive polymers is pretty straightforward and flexible in processing. Pyrrole, thiophene, and dopamine are used as photoactive monomers in the biosensing event [25].

The cavities' affinity to templates is related to the selection of monomers. The choice of the monomer can be performed experimentally or using a molecular simulation design assisted with a computer program. The simulation-based applications make easy selection of monomer and solvent, which leads to the easy fabrication of MIP-based sensors.

Methacrylic acid (MAA), polythiophene, polypyrrole (PP), poly(3,4- ethylenedioxythiophene), o-phenilendiamine (o-pD), polyvinylpyrrolidone (PVP), polyamine (PA), polyaniline and dimethylamino ethyl methacrylate (DMAEMA) are typically used as functional monomer for electropolymerization.

The interactions with functional groups are provided with non-covalent, electrostatic, covalent, semi-covalent, and metal-mediated. Template molecules bind with functional monomer via hydrogen bonding or van der Waals interactions, electrostatic or ionic interactions, a covalent bond, and ligand-metal or metal-ligand coordination. If needed, the functional group mixes with a suitable cross-linker. After electropolymerization, the imprinted target molecule is removed from the polymeric matrix through washing, cleavage of chemical bonds, or ligand exchange. The polymeric matrix, including cavities specific to the target molecule, should recognize the target without non-specific interactions.

The MIP system has been employed as a bio-mimic polymer by recognition ability towards the omics, as described by J.A. Ribeiroa et al. [29]. Toluidine Blue is a member of the water-soluble azine-type redox dye family. It is an electronic mediator in constructing metabolomics MIP-based biosensors for biosensing applications due to their unique chemical and electrochemical properties. Using MIP systems, the authors developed a MIP biosensor for detecting CA 15-3 biomarker from 0.10 U mL^{-1} to 100 U mL^{-1} with LODs of <0.10 U mL^{-1}. Furthermore, the suggested MIP sensor was applied to selective and sensitive target CA 15-3 determination in spiked artificial serum samples, using the DPV technique.

3.1.3. Cross linker

The cross-linker is one of the critical components for designing a MIP sensor. For enhancing the performance of the MIP-sensor, the ratio of monomer/cross-linker should be optimized. Before fabrication, the best cross-linker and monomer may have been selected via combinatorial and computational analysis. The hydrophilic monomers and cross-linkers are preferred to obtain the suitable MIP sensor in aqueous samples because of their more excellent compatibility with aqueous environments.

Moreover, the cross-linker is used to ensure the long-term stable morphology of binding sites. Compared to traditional MIPs, the template is easily removed/recombined from polymeric structure included with cross-linker. The widely used cross-linkers are trimethylolpropane trimethacrylate (TRIM), tetramethylene dimethacrylate and ethylene glycol methacrylate (EGDMA), and divinylbenzene (DVB) in non-covalent imprinting [28].

Cross-linker is mainly utilized for the biosensing of disease biomarkers. As an example, Reddy K.K. et al. reported that the electrochemical MIP-based sensor was developed for creatinine analysis [30]. The polymeric matrix is prepared with the functional monomer methyl acrylate and cross-linker ethylene glycol dimethacrylate in the presence of the template creatinine. The polymeric mixture was heated at 60−70°C for 24 h. To remove the creatinine from the polymeric matrix, the polymer was washed sequentially with firstly distilled water, then methanol, and 1 M HCl each for 120 min. The mixture of the polymeric matrix and graphite powder has filled the hollow of the carbon paste electrode. The surface characterization was performed with RAMAN and scanning electron microscopy (SEM). The analytical performances were evaluated with the EIS technique. They found that cross-linker increased the stability, sensitivity of metabolites.

3.1.4. Extraction methods

The MIP systems are basically based on electropolymerization consisting of monomer and template, subsequent extraction of the template from the polymer, and binding the target to obtained cavities specific to the target. The cavity is formed according to the size, shape, and functionality of the template. The morphology of cavities is important factor for specific recognition between MIPs and metabolites.

The interaction between MIPs and metabolite provides the non-covalent, semi-covalent, and covalent bonding. The non-covalently interaction, including van der Waals forces, hydrogen bonding, and dipolar interactions, widely occurs in MIP fabrication because of its excellent adaptability [21].

The covalent binding occurs in the formation of the template-containing bulk polymer. In the extraction step, the covalent bonding between template and monomer must be cleaved. Following cleavage of binding, the target is rebound to the polymeric matrix's binding site through reversible covalent binding or non-covalent interactions. The covalent binding provides a more homogeneous distribution and a greater density of binding sites. When rebinding of template, adjustment of specific recognition sites and a higher binding constant can be cited as advantages of covalent bonds. On the other hand, more aggressive solvents are utilized to extract the target from the polymeric matrix. In this case, the solvent can ultimately damage the covalent binds [31].

Different treatments can be used to remove the target from the polymeric matrix, such as the pH or ionic strength of the solution, temperature, surfactants, electrode types, or ultrasonication.

After the extraction process, a small part of the template may remain in the polymeric matrix. So, the residual of template molecules can create a problem for the rebinding process. In this case, the quantitative analysis of the target can be limited. Therefore, the selection of extraction solution is a critical parameter for the fabrication of MIP-sensor [32].

The porogenic solvent affects the interaction between target and monomer. It behaves as dispersion media and contributes to the forming homogeneous cavity during the polymerization reaction. The toluene, acetonitrile, and chloroform solutions are widely used in non-covalent imprinting [31].

Mao Y. et al. reports that the electrochemical MIP nanosensor was developed for the determination of dopamine. Novel graphene-MIP composite is used as a recognition element. The recognition element was synthesized through free radical polymerization (FRP) [33]. The MIP sensor was obtained with two steps: Firstly, the dopamine was adsorbed in the graphene-MIP composite surface. Then, MAA as functional monomers and ethylene glycol dimethacrylate (EGDMA) as cross-linker were used for polymerization. For removal of the template, a potential scanning technique was performed. Therefore, dopamine could be rapidly and completely removed from the polymeric matrix. This technique has many advantages: faster desorption, adsorption dynamics, higher selectivity, and binding capability.

3.2. The effect of using nanomaterials

Nanotechnology provides excellent contributions to the fabrication of biomimetic systems. In recent years, the application of nanoparticles as sorbent has gained significant attention in the bioanalysis field. Furthermore, the combination with nanomaterials and rational designs enhance the performance of MIP-based biosensor. Therefore, MIP-based nano biosensors have many medical applications (sensor design and POCT) [28].

The structures of graphene oxide, carbon nanotubes, or nanoparticles are more suitable for MIP design. This is because nanomaterials enhance the surface area of MIP cavities. Consequently, the target molecule easily binds to the active site of the polymeric matrix, and the lower concentration of target reaches. In this case, the sensitivity of the MIP nano biosensor can be enhanced because of its binding capability.

MIPs combined with nanomaterials enhance the performances of electrochemical biosensors because of nanomaterials' electrical, catalytic properties and excellent conductivity [21]. Noble metal nanoparticles (such as AuNPs, AgNPs, PtNPs, PdNPs) and metal oxide nanomaterials (such as TiO_2, Fe_2O_3, etc.) present various important advantages [21].

There are many nanomaterial modification techniques for MIP electrodes. The simple and easy method is that deposition and spin coating on MIP electrode. The thickness and porosity of polymer can be controlled by the spin-coating technique [34]. Self-assembled monolayers (SAMs) are widely utilized for the fabrication of MIPs nanoparticles. The method consists of two steps: Firstly, Nanoparticle imprinted polymer is prepared; then, the obtained MIPs nanoparticles are assembled to the modified electrode using SAM technique via the covalent bond. The monomer is applied on electrode surface using electropolymerization or UV Light-Induced Polymerization technique. The polymer template is formed by electropolymerization using the CV technique. The thickness of the polymeric film can be easily changed with an optimized CV scan. The electrode surface is characterized by electrical impedance spectroscopy (EIS) and CV techniques. As the polymer layer thickens, the charge-transfer resistance of the surface will be increased. Moreover, the nanomaterials are used as a solid substrate to immobilize the target arranged in a specified orientation before polymerization.

The MIP-based biosensor has been reported for cholesterol using multi-walled carbon nanotubes (MWNTs) and Au nanoparticles (AuNPs) [35].

p-Aminothiophenol (P-ATP), $HAuCl_4$, tetrabutylammonium perchlorate (TBAP), cholesterol were assembled on the surface of the modified GCE by an electropolymerization with a potential range from −0.3 to 1.2 V (scan rate 50 mVs^{-1}). The cholesterol molecule is removed from the polymeric matrix through immersing the ethanol/water (4:1,v/v) solution presenting 0.5 M HCl. The prepared p-ATP/AuNPs−MWNTs/GCE is an innovative MIP transducer for application in clinical diagnostics of cholesterol with high sensitivity and excellent stability. The linear response range of the MIP sensor was from 1×10^{-13} to 1×10^{-9} M, and the LOD was 3.3×10^{-14} M for the detection of cholesterol.

4. Advanced technical application of MIPs at biomarkers

MIP-based nanosensors used in the analysis of some metabolites and their related parameters are summarized in Table 1. The table refers to biomarkers, diseases, techniques, electrodes monomers, nanoparticles, linear ranges, LOD/LOQ, and samples.

Paratea K. Et al. demonstrate an electrochemical cotinine MIP sensor with ortho-phenylenediamine (oPD) monomers to produce a MIP-based screen-printed carbon electrode (SPCE) covered with graphene and PtNPs [36]. The sensitivity level of the modified sensor enhances 4-fold. CV was successfully conducted in a wide sensing range of 1−100 nM as an effective detection technology for cotinine measurements with a LOD of 0.33 nM in ferricyanide solution. In addition, an accurate cotinine concentration is detected in saliva for non-smokers and smokers and in tobacco products within 12 min. The authors referred that the developed biosensor is well-suited to deliver through POC testing.

In chiral sensing, Pandey I. et al. used carbon dots as prominent nanomaterials due to their inherent electronic properties, electrocatalytic properties, enormous surface area-to-volume ratio, and particular enhanced surface area for ultrasensitive and selective detection of D-Ascorbic acid (D-AA) and L-Ascorbic acid (L-AA) [37]. The polyaniline/pencil graphite electrode (PANI/PGE) was used as a platform for non-covalently attaching substituted groups of the L-AA and D-AA molecules. The suggested electrochemical MIP chiral sensor has a linear range of 6.0−165.0 nM and 6.0−155.0 nM and a LOD of 0.001 nM and 0.002 nM for L-AA and D-AA molecules, respectively. The chiral detection of L-ascorbic acid was successfully evaluated in pharmaceuticals and human serum samples. Moreover, the results of human plasma samples with pregnant and non-pregnant were compared.

A sensitive on-off ratiometric electrochemical for quantitative detection of dopamine was described [38]. The bare gold electrode was electrodeposited with porous gold nanoparticles (NPG) utilizing a signal amplification. NPG was prepared with the first electrodeposition of mixture solution of $HAuCl_4$ and $CuSO_4$ in 0.5 M H_2SO_4, then dealloying Cu. In MIP sensing, the electroactive polymer made from electropolymerization of thionine can be used as a target molecule recognizer. The thionine-based NPG electrode was conducted by electropolymerization of thionine by CV from −0.4 V to 0.4 V (scan rate of 0.1 V s^{-1}) for 30 consecutive cycles. After obtaining the MIP platform, the polymeric matrix was exposed to a removal solution for creating the cavity specific to the target molecule. In the dopamine binding process, the dopamine occupies some of the cavities in the polymeric matrix. Using the DPV technique, the proposed sensor exhibited good sensitivity in the linear monitoring range of 0.3−100 μM with detection limits of 0.1 μM. Moreover, the developed sensor was directly tested for dopamine concentration in the cerebrospinal fluid (CSF) sample.

For the detection of 17β-estradiol, a nano complex consisting of Fe_3O_4 nanobeads/reduce graphene oxide nanostructure as the glassy carbon electrode modifier and MAA and DVB as the monomer was used [39]. Due to the roles played by the two nanomaterials, the sensing platform has shown important synergetic electrochemical performance: Fe_3O_4 nanobeads have boosted the surface-to-volume ratio and the reduced graphene oxide nanostructure have amplified the electrochemical response. The MIP is prepared with the fragmentation chain transfer (RAFT) polymerization technique. A RAFT polymerization technique exhibits high affinities toward target molecules, and it is utilized to prepare more homogeneous structures with improved distribution of imprinted cavities. The MIP biosensor has been employed successively to test water samples, showing a wide linear range of 0.05−10 mM and a LOD of 0.819 nM, respectively.

Martinset G.V. et al. developed an ultrasensitive electrochemical biosensor based on a MIP layer prepared by bulk polymerization of phenol on gold electrode surface for electrochemical detection of 8-hydroxy-2′-deoxyguanosine (8-OHdG) [40]. The proposed electrochemical biosensor showed high selectivity and effectively detected 8-OHdG with a 0.74 pg mL^{-1} detection limit in urine samples. For characterization, the film of MIP is displayed by confocal microscopy by means of the FITC-anti-8-OHdG. Furthermore, RAMAN spectroscopy, FTIR, and SEM confirmed the formation of a polyphenol thin film on the gold electrode.

Table 1
MIP determination of selected biomarkers.

Biomarker	Disease	Technique	Electrode	Monomer	Nanoparticle	Linear Range	LOD/LOQ	Sample	References
Cholesterol	Clinical diagnostics of cholesterol	MIP film-based electrochemical sensor	GCE	P-ATP, TBAP	MWNTs-AuNPs	1×10^{-13} −1×10^{-9} M	3.3×10^{-14} M	Biological samples	[35]
Cotinine	Nicotine	MIP film-based electrochemical sensor	SPCE	o-PD	Graphene/platinum	1−100 nM	0.33 nM	Saliva swab sample	[36]
Creatinine	Muscle contraction	MIP film-based Impedimetric sensor	CPE	Methylacrylate	–	0.18−5.9 μM	20 ng mL^{-1}	Human serum and artificial urine	[30]
Creatinine	Skeletal muscles	MIP film-based electrochemical sensor	Au-SPE	PVC-COOH	–	0.1−1 μg/mL	0.016 ng/mL	Human urine	[53]
D-Ascorbic acid and L-Ascorbic acid	Preeclampsia	MIP film-based electrochemical sensor	Pencil graphite electrodes	Aniline ferrocenesulfonic acid-C-dots	C-dots	6.0−155.0 nM and 6.0−165.0 nM	0.001 nM and 0.002 nM	Pharmaceuticals and human plasma	[37]
Diosgenin	Diabete	MIP film-based electrochemical sensor	GCE	pABA	–	0.003−0.13 mM	8.95×10^{-4}mM/ 2.98×10^{-3}mM	Human plasma and serum	[54]
Dopamine	Neurological diseases	MIP film-based ratiometric sensors	Au electrode	pThi	–	0.3−100 μM	0.1 μM	Artificial cerebrospinal fluid	[38]
Dopamine	Neurological diseases	MIP film-based electrochemical sensor	GCE	Pyrrole	MWNTs-COOH	6.25×10^{-7} −1×10^{-4} M	6×10^{-8} M	Biology samples	[55]
Dopamine	Neurological diseases	MIP film-based electrochemical sensor	GCE	MAA	GSCR	1.0×10^{-7} −8.3×10^{-4}M	1.0×10^{-7}	–	[33]
17β-estradiol	Natural manure-borne hormone	MIP film-based electrochemical sensor	GCE	MAA	Fe_3O_4-RGO	0.05−10 mM	0.819 nM	Water	[39]
17-β Estradiol	Mammalian female development	MIP film-based electrochemical sensor	Au electrode	p-ATP	–	3.6 fM-3.6 nM	1.09 fM	River samples	[56]
17-β Estradiol	Sex hormone	Molecularly imprinted polymeric microspheres	SPCE	MAA	MWNTs-AuNPs	1×10^{-15} −1×10^{-16} M	2.5×10^{-16} M	Fish serum samples	[57]
8-hydroxy-2′-deoxyguanosine(8-OHdG)	Oxidative stress	MIP film-based electrochemical sensor	Au electrode	Phenol	–	0.1−100 pg/mL	0.74 pg/mL	Human urine	[40]
5-hydroxyindole-3-acetic acid (5-HIAA)	Carcinoid tumor	Artificial enzyme-based catalytic sensor	Disposable SPEs	4-VPY	–	1−50 μM	1.4 μM	Human urine	[41]
Lactate	Tissue oxygenation	MIP film-based electrochemical sensor	SPE	3-APBA	Ag nanowires	10^{-6} -0.1 M	0.22 μM	Human sweat	[42]
Lactate	Hypoxia	MIP film-based Impedimetric sensor	Planar screen-printed	3-APBA	–	3 mM−100 mM	1.5 mM	Human sweat	[43]
Lactate		MIP film-based electrochemical sensor	GCE	o-PD	RGO)-AuNPs	0.1 nM−1.0 nM	0.09 nM	Sugarcane vinasse sample	[44]
L-cysteine	Immune system cells	MIP film-based electrochemical sensor	CPE	MAA	–	2×10^{-8} −18×10^{-8} M	9.6 nM	Tap water and human blood plasma samples	[45]
N-hexanoyl-L-homoserine lactone	Gram-negative bacterial infection	nano-MIP Quartz crystal resonator (QCR) sensor	–	MAA	–	–	1 μM	–	[58]
3-nitro-L-tyrosine (3-NT)	Oxidative stress	Amperometric sensor	SPE	–	$CdWO_4$-RGO	18.5 nM−1.84 mM	3.24 nM	Human serum and urine	[46]
3-nitrotyrosine	Oxidative stress	MIP film-based electrochemical sensor	GCE	Pyrrole	Activated MWCNT-GONRs	0.2−50.0 mM	50.0 nM.	Human serum and urine	[47]
3-nitrotyrosine	Oxidative stress	MIP film-based electrochemical sensor	SPCE	Phenol	–	500 nM−1 mM	22.3 nM	Human urine	[59]
Progesterone	Reproductive hormone	MIP film-based electrochemical sensor	–	Aniline-co-metanilic acid	–	–	1.0 pg mL^{-1}	Human urine	[48]
Pyruvic acid	Cancer	MIP film-based enzyme-free sensor	CPE- CNT-CPE	MAA	CNT	0.1−200 μM	0.048 μM	Plasma and urine	[9]
Sarcosine	Prostate cancer	MIP film-based impedimetric sensor	AU-SPE	p-ATP	–	0.011−17.900 μM	8.5 nM 1.0 ng/mL	Human urine	[49]

(*continued on next page*)

Table 1 (*continued*)

Biomarker	Disease	Technique	Electrode	Monomer	Nanoparticle	Linear Range	LOD/LOQ	Sample	References
Sarcosine	Prostate cancer	MIP film-based potentiometric sensor	CPE	MAH	–	10^{-2}–10^{-6} mM	1.35×10^{-7} mM	Human urine	[50]
Sarcosine	Prostate cancer	MIP film-based electrochemical sensor	CPE	MAA	–	5.0 μM–1.1 mM	0.38 μM	Human urine	[51]
Sarcosine	Prostate cancer	MIP film-based for Solid-Phase Extraction (SPE)	–	MAA	–	–	–	Human urine	[60]
Sarcosine	Prostate cancer	MIP film-based electrochemical sensor	Au electrode	MAA	Fe_3O_4-zeolitic imidazolate framework-8	1–100 pM	0.4 pM	Human urine	[52]
Sarcosine	Prostate cancer	MIP film-based electrochemical sensor	SPE	2-acrylamido-2-methyl-1-propanesulfonic acid - 4-vinylpyridine	MWCNT-Nafion®-$Ni(OH)_2$	3.2–25.0 μM	0.96 μM	Human urine	[61]

Glassy carbon electrode: GCE, screen-printed carbon electrode: SPCE, carbon paste electrode: CPE, screen-printed gold electrodes: Au-SPE, Screen-printed electrodes: SPE, p-aminothiophenol: P-ATP, tetrabutylammonium perchlorate: TBAP, ortho phenylenediamine: o-PD, carboxylic polyvinyl chloride: PVC-COOH, p-aminobenzoic acid: pABA, polythionine: pThi, methacrylic acid: MAA, 4-vinylpyridine: 4-VPY, 3-aminophenulboronic acid: 3-APBA, methacryloylamido histidine: MAH, multi-walled carbon nanotubes: MWNTs, Au nanoparticles: AuNPs, graphene sheets/congo red: GSCR, reduced graphene oxide: RGO, graphene oxide nanoribbons: GONRs, carbon/carbon nanotube: CNT.

Antuña-Jiménez D. et al. have designed a selective and sensitive MIP-based electrochemical sensor to determine 5-hydroxy indole-3-acetic acid (5-HIAA), the primary metabolite of serotonin. The authors used magnetic-core/porous-shell molecularly imprinted composites as the recognition phase. The sensor exhibited a significant increase in selectivity and avoided many unspecific matrix effects in determining the 5-HIAA. Moreover, under optimized conditions, the calibration curve was obtained linearly in the concentration ranges of 1–50 μM (with the detection limit of 1.4 μM). This sensor is compared with HPLC, and the results show that the developed sensor is suitable for the analytical diagnosis of carcinoid tumors [41].

In Fig. 4., Zhang Q. et al. initiated an electrochemical sensing technique for the extremely sensitive and specific lactate determination in the human sweat [42]. To this purpose, the Ag Nanowires (AgNWs) were coated on the screen-printed carbon electrode before electropolymerization of 3-aminophenyl boronic acid (3-APBA). As a result, the authors created a wearable electrochemical biosensor for the non-invasive monitoring of sweat lactate on human skin during exercise. The MIPs-AgNWs biosensor revealed high sensitivity and specificity for the detection of lactate from 10^{-6} M–0.1 M, with the detection limit of 0.22 μM. The developed wearable sensor was proved by successfully detecting lactate in the military, sports, and biomedical fields [42].

In another lactate study, the non-enzymatic sensor was developed using a screen-printed carbon electrode. The monomer of 3-aminophenylboronic acid (3-APBA) is applied by electropolymerization. The linear range of 3 mM–100 mM and LOD of 1.5 mM were obtained by impedimetric electrochemical technique. The storage stability of the developed sensor was 6 months. The future perspective of this study is to use it in non-invasive clinical analysis and sports medicine [43].

Pereira T.C. et al. reports that the lactic acid was developed MIP-based nanosensor. Reduced graphene oxide and gold nanoparticles were used as modification agents due to enhancing the electrode performance. MIP is obtained by means of electropolymerization of o-phenylenediamine (o-PD) containing lactate. Nanomaterials modified MIP electrode is characterized with CV, EIS, SEM, and atomic force microscopy. The linear range and LOD were obtained as 0.1–1 nM and 0.09 nM, respectively [44].

An electrochemical sensing strategy comprising a methacrylic acid (MAA) modified carbon paste electrode (CPE) has been reported to determine L-cysteine by Aswini et al. The proposed electrochemical sensor showed a linear response for L-cysteine in the concentration range of 2×10^{-8} to 18×10^{-8} M with a detection limit value of 9.6 nM. Furthermore, under the optimal experimental conditions, L-cysteine (Cys) in tap water and human blood plasma samples were quantitatively analyzed with reproducible results [45].

The $CdWO_4$ nanodots decorated reduced graphene oxide (CdWO4 ND@RGO) nanocomposite was drop casted on the screen-printed carbon electrode. The suggested electrochemical sensor has a linear range of 18.5 nM–1.84 mM and a LOD of 3.24 nM. The developed immunosensor was also utilized to evaluate a human serum and urine, yielding accurate and precise 3-nitro-L-tyrosine detection results. Electrochemical biosensors have taken a lot of attention because of their abilities, such as fast response, high recovery, and low-cost instrumentation [46].

Graphene nanoribbons (GNRs) are thin elongated strips of graphene. GNRs have a suitable structure to mix with aromatic molecules via p-p interaction, different from graphene nanosheets (GNs) and carbon nanotubes (CNTs). In this regard, a sensitive MIP-based electrochemical biosensor for quantitative detection of 3-nitrotyrosine (3-NT) was described utilizing a signal amplification method. The graphene oxide nanoribbons (GONRs) were produced

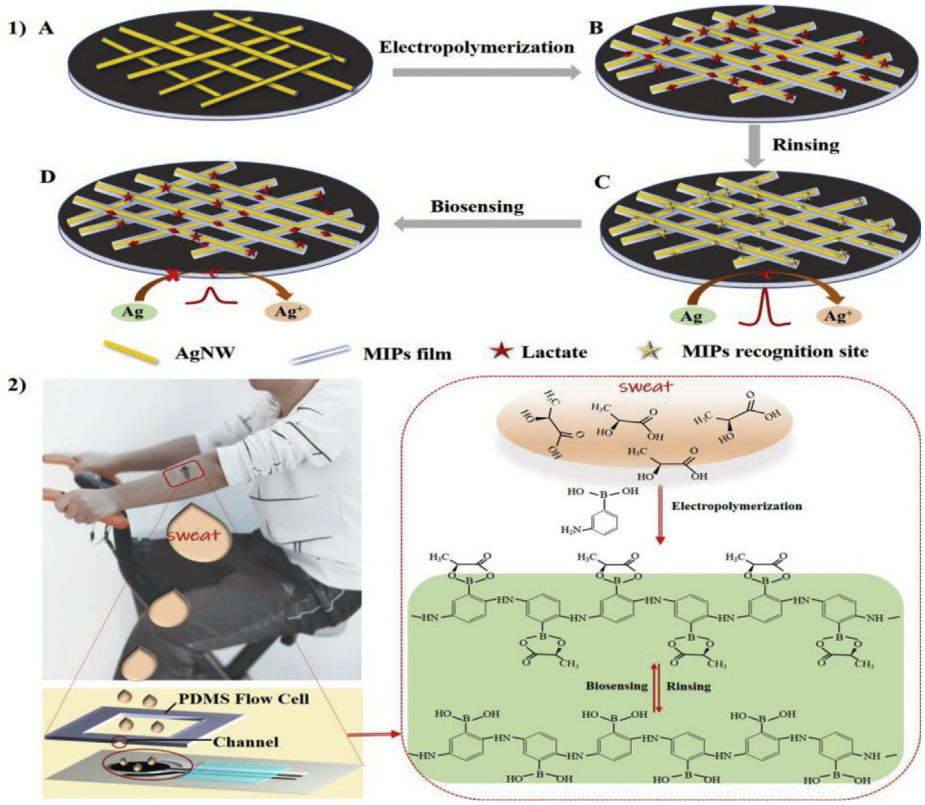

Fig. 4. (1) The figure shows the fabrication of the MIPs-AgNWs electrochemical biosensor for the epidermal monitoring of lactate. (A) AgNWs modified electrode (B) AgNWs/MIP modified electrode (C) Removal of lactate from AgNWs/MIP modified electrode (D) Lactate binding AgNWs/MIP modified electrode in PBS solution or human sweat. (2) The application of AgNWs/MIP/SPCE on a male volunteer's arm. Reproduced with permission from Elsevier [42].

using an activated multiwall carbon nanotube (AMWCNT). The pyrrole is used as a monomer to electropolymerized MIP doped with AuNPs. The polymeric matrix, consisting of AuNPs, monomer, and template (ratio of: 0.5:0.5:0.5), is the best selected MIP fabrication. The $K_3Fe(CN)_6$ was used as a probe to evaluate each fabricated step. The developed MIP-based biosensor exhibited good selectivity, stability, and sensitivity for 3-nitrotyrosine detection under optimum conditions. Furthermore, a linear correlation was established between electrochemical signals, with a detection limit of 50 nM, and the 3-nitrotyrosine concentration between 0.2 μM and 50.0 μM. Moreover, the proposed MIP-based biosensor was successfully applied for highly selective, reproductive, stable, and satisfactory detection of 3-NT in human serum and urine samples [47].

Lee M-H. et al. have developed a molecularly imprinted poly(aniline-co-metanilic acid)s (p(ANI-co-MSAN)) sensor for sensitive determination of progesterone [48]. The electrochemical results showed that the sensor has high sensitivity and selectivity for progesterone with detection limit values of less than 1.0 pg mL^{-1} for progesterone in urine samples. The sensor was successfully applied to the direct determination (0.64–5.27 ng mL^{-1}) of progesterone in random urine analysis, and the proportional result was obtained with a commercial ARCHITECT system. Moreover, the morphology of progesterone-imprinted p(ANI-co-MSAN) sensors was characterized by SEM.

To obtain MIP/CNT-CP electrode, the molecule polymeric imprinted electrode is fabricated with the modification of a carbon/carbon nanotube paste electrode with MWCNT. MIP contributes to enhancing the electro-exchange rate and pre-concentrated molecule to modified electrode surface [9]. EIS was successfully conducted in the linear concentration range of 0.1–200 μM for Pyruvic acid (PA) measurements with a detection limit of 0.048 μM. In addition, an accurate PA concentration may be detected by the MIP/CNT-CP in plasma and urine samples. The represented RSD values of 3.6% are good evidence for the validity of the method.

The SPCE was first modified by in situ deposition of AuNPs using a CV method in Fig. 5. Then, these AuNPs/SPCEs were used for MIP electropolymerization. MIP electrochemical platforms have been created for identifying Sarcosine by use of the gold-nanoparticles and poly-amino thiophenol (p-ATP) layers by Nguy T.P. et al. [49]. For evaluating the selectivity of sarcosine detection, L-alanine and L-lysine were discriminated. The obtained results show that it is the first proof-of-principle for the MIP platform. As a result, electrochemical signals are increased with differing quantities of target Pyruvic acid (PA). The proposed sensor exhibited good sensitivity in the liner monitoring range of 0.011–17.9 μM with detection limits of 8.5 nM.

Ozkutuk E.B et al. fabricated a molecularly imprinted methacryloylamido histidine (MAH) sensor for simple and rapid analysis of Sarcosine [55]. The ethylene glycol dimethacrylate (EDMA) was used for cross-linking. The electrochemical results showed that the 2D MIP sensor has high sensitivity and selectivity for Sarcosine with detection limit values of 1.35×10^{-7} mM. The sensor was successfully applied to the direct determination (10^{-2}–10^{-6} mM) of Sarcosine by potentiometric sensor. The storage stability was found as >5.5 months [50].

A rapid and selective electrochemical sensing strategy comprising a methacrylic acid (MAA) modified carbon paste electrode (CPE) has been reported to determine Sarcosine by Sheydaei O. et al. The proposed electrochemical sensor showed a linear

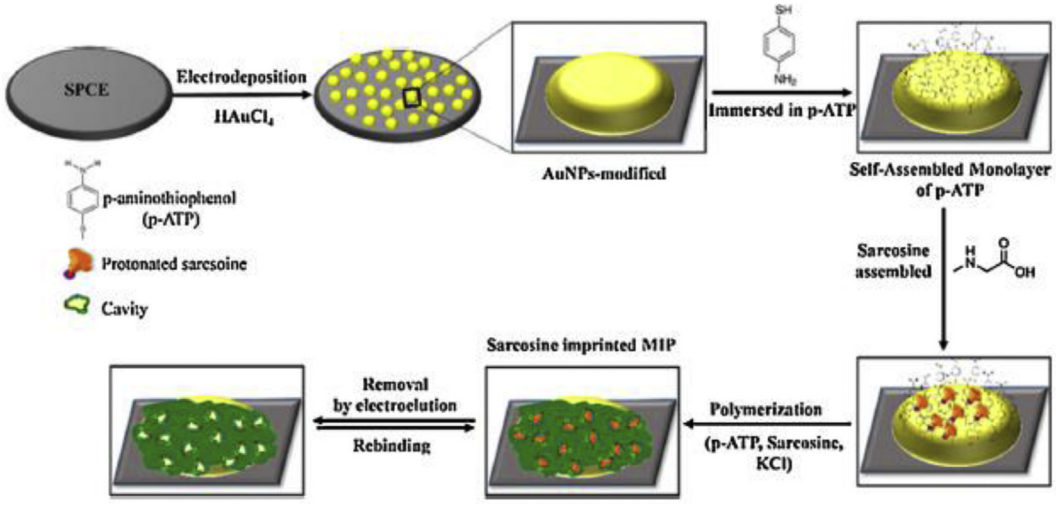

Fig. 5. Work chart of preparation sensor chips using AuNPs/SPCE. Reproduced with permission from Elsevier [49].

response for Sarcosine in the concentration range of 5.0 μM−1.1 mM with a detection limit value of 0.38 μM. Furthermore, under the optimal experimental conditions, Sarcosine in urine samples of healty patients and cancer patients were quantitatively analyzed with reproducible results of 2.1% [51].

The super-magnetic metal-organic frameworks (MOFs) are zeolite-like compounds synthesized by metal ions and chelated clusters of organic ligands. In this regard, a sensitive MOF@MIP-based electrochemical biosensor for selective and sensitive detection of Sarcosine was described utilizing cyclic voltammetry. First, the functional monomer MAA and cross-linker EGDMA were electropolymerized to the electrode surface. Then, MOF@MIP was self-assembled onto Au electrode through magnetic interaction to obtain the sensing unit of the electrochemical sensor. The imprinting factor was found as 22.0. The suggested electrochemical MIP sarcosine sensor has a linear range of 1−100 pM and a LOD of 0.4 pM in urine samples [52].

5. Conclusions and future research

In recent years, the development of high-sensitivity MIP-based electrochemical sensors has played an essential role in analyzing and evaluating metabolites. Electrochemical methods are preferred due to their advantages, such as being more sensitive, selective, low cost, and environmentally friendly than other analytical methods. Synthetic materials such as MIPs that provide analyte-specific selectivity on the electrode surface using different monomers and cross-linkers and commercially used nanomaterials have contributed significantly to the high-performance electrochemical sensing platform. In this review, MIP-based electrochemical applications for the analysis of selected metabolites are summarized. When the studies in the literature are examined, nanomaterials with high conductivity and large surface area and the combination of MIPs yielded more sensitive and more selective results in the analysis of metabolites.

In the future, studies on the development of disposable sensors for accurate and rapid detection of metabolites are expected to increase in the medical field and clinical studies. As precise analysis of metabolites is difficult due to the complexity and diversity of metabolic pathways, it is anticipated that research and design of portable MIP-based electrochemical sensors will increase in the future to accelerate the medical diagnosis and treatment process. Moreover, with the development of more reliable MIP-based sensors and the appropriate design of multiple detection systems, many metabolites will be detected simultaneously. These data will guide clinicians in the diagnosis and treatment of the disease.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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