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Abstract
In this chapter we discuss molecular imprinting technology (MIT), molecular imprinted polymers (MIPs), and their compatibility on a proper transducer to construct a sensing system. Molecularly imprinted sensors (MISens), in other words, artificial receptor-based sensors synthesized in the presence of the target molecule, are capable of sensing target molecules by using their specific cavities and are compatible with the target molecule. This MIP technology is a viable alternative of artificial receptor technology, and the sensor technology is capable of detecting any kind of molecule without pre-analytic preparations. In this chapter, you can find examples, sensor construction techniques and fundamentals of MIP and sensor combinations to look forward in your studies. For sensor technology, we explained and discussed the new sensing technologies of MIP-based electrochemical, optical (especially surface plasmon resonance, SPR), and piezoelectric techniques. Therefore, this chapter presents a short guideline of MISens.

Keywords: molecular imprinting, sensors, artificial receptors, impedimetric sensors, capacitive sensors, potentiometric sensors, amperometric sensors, fluorimetric sensors, SPR sensors, QCM sensors, piezoelectric sensors

1. Introduction
Nowadays, technology develops exponentially and the rate of article publishing and patent applications are immensely high reflecting the growth of technological improvements and
discoveries. The purpose of all developmental statuses is to simplify human life. Therefore, every system is being designed in an “all in one fashion” for devices and equipment. You can imagine, for instance, that if we compare the current mobile phone systems with 10 years earlier, we will see that there is a big jump. This technological development has a wide area of application ranging from health to food applications, from environment to space. The best providence of this technology is its ability to provide people a simpler life, which comes within the simplified new treatment systems for medicine, or with the control of the environmental balance more easily by simple devices. These simple devices are being developed especially for personal use such as chemical sensors and biosensors. These devices are famous for their features such as that they are easy to use, cost-effective with a high sensitivity and selectivity. Therefore, sensor technology is widely used in different platforms ranging from health technologies to environmental analysis methods [1-6]. Generally, sensors consist of three parts: transducers, recognition elements, and an analytical device. Transducers are part of sensors which convert energy from one form to another. For example, piezoelectric transducers convert electrical charges produced by piezoelectric solid materials into energy. Recognition elements (enzyme, DNA, antibodies, etc.) and a proper analytical device to show noticeable signals formed by transducers are the elements of chemical and biochemical sensors [7-11]. Chemical sensors use chemically formed materials (nanomaterials, MIPs, etc.) as recognition elements, whereas biochemical sensors use biomolecules (enzymes, antibodies, DNAs, receptors, proteins, etc.) as recognition elements. If we compare these sensor types, although biosensors are more selective then chemical sensors, chemical sensors have advantages such as the capacity to resist harsh conditions such as strong pH, extreme ionic strength, and a wide variety of organic solvents. In this chapter, we especially point out these differences together with the concepts of construction of molecularly imprinted sensors. Just like biosensors, the chemical sensors are divided into two main functional combinations: affinity-based sensors and catalytic sensors. Catalytic sensors are modified with different molecules that show catalytic properties [12]. Affinity-based sensors, which are MIP-based sensors, have a specific recognition pattern mechanism of recognition for the target analyte which mimics to recognize target analyte [13].

2. Molecular imprinting technology

In brief, molecular imprinting is defined as the formation of artificial receptors for a specific target molecule on a polymer or on self-assembled materials. Natural receptors are widely used for sensor technology to target the analyte, leading to electrochemical, optical, and mass or magnetic changes on transducers [14-18]. MIPs are obtained by polymerization of a monomer and a cross-linker, which are located around the target molecule (Fig. 1). This assembly of a monomer around a target molecule is encouraged by covalent and non-covalent interactions. It is easier to remove the target molecule from a non-covalently formed MIP-target molecule complex than removing from a covalently formed MIP-target molecule complex. Just as we described, MIPs are synthetic polymers, which can only be used as plastic antibodies for now. It means that, currently, MIPs on sensors are only used as affinity sensors and not as a catalytic biochemical enzyme mimicking sensors.
Molecular imprinting technology, in general, is developed as bulk polymerization (3-D) and surface imprinting polymerization (2-D). Bulk polymerization is prepared as bulk materials, so they have further preparation steps before use to recognize desired materials, for example, grinding of bulky MIPs, which cause disruptive heterogeneous binding sites leading to poor site accessibility. Moreover, there are embedded target molecules inside the bulk polymers [19]. Surface imprinting is more advantageous due to controlled surface imprinting and its convenience for sensor technology. For the sensor and the analytical device preparation by using MIPs, there are two methods to design MIPs; one is an in situ technique that imprinted polymer is prepared on the transducer, whereas the other is an ex situ technique that imprinted polymer is prepared separately from transducers, where MIPs are immobilized on transducers after this preparation to construct MISens.

3. Sensors

Chemical sensors are a major class of sensors, which have many applications, such as environmental and food analysis, process control, and medical diagnosis. A chemical sensor is a device that transforms chemical information, ranging from the concentration of a specific sample component to the analysis of the total composition of a sample, into an analytically useful signal [20] and [21]. Like many fields in science, chemical sensors have benefited from the growing power of computers, integrated electronics, new materials, novel designs, and processing tools. Breakthroughs over the last decade have pushed chemical sensors into new markets, as well as to new applications within existing markets [22].

When operated, a chemical sensor performs two functions: recognition and transduction. First, the analyte interacts in a more or less selective way with the recognition (or sensing) element, which shows affinity for the analyte. The sensing element may be composed of distinct molecular units called recognition receptors. Alternatively, the recognition element can be a
material that includes certain recognition sites in its composition. Beyond this, the recognition element can be formed of a material with no distinct recognition sites, but capable of interacting with the analyte. In a chemical sensor, the recognition and transduction functions are integrated within the same device. An analytical device with no recognition function is not a chemical sensor but a concentration transducer [20, 23]. The signal from a sensor is typically electronic in nature, being a current, voltage, or impedance/conductance change caused by the change in analyte composition or quality. While chemical sensors contain a physical transducer and a chemically sensitive layer or recognition layer, the micro-instrument or spectrometer sends out an energy signal, which can be thermal, electrical, or optical, and reads the change in this same property caused by the intervening chemical and this is close to molecular spectroscopy [24].

Biosensors have specific recognition elements of the proper chemical substances, which is performed as an analytical devise. The biological material that serves as recognition element is used in combination with a transducer. The transducer transforms the concentration of substrate or product to electrical signal that is amplified and further processed. The biosensors may utilize enzymes, antibodies, nucleic acids, organelles, plant and animal tissue, whole organism, or organs. Biosensors that contain biological catalysts (enzymes) are called catalytic biosensors. These types of biosensors are the most abundant, and they have their largest application area in medicine, ecology, and environmental monitoring [25-27].

Molecularly imprinted polymers (MIPs) are synthetic materials used as recognition elements in the design of sensors due to their higher thermal stability than biological receptor, reusability, and selectivity compared to biological receptors. These polymeric materials bind to the target molecules causing variations in physical parameters, such as mass, absorbance, or refractive index depending upon the shape, charge, and functionality of the target molecule leading to Ref. [28]. The design of these synthetic materials, which are able to mimic the recognition processes found in nature, has become an important and active area of research making molecular imprinting one of the strategies followed to create materials with recognition ability comparable to the natural systems in recent years.

4. Molecularly imprinted sensors

A combination of molecularly imprinted polymers and transducers form a synergistic device. Just as we mentioned before, MIP’s have the ability to resist pH, organic environment, and ionic strength. Therefore, their usage in sensor technology is very beneficial. Because of this, studies including molecular imprinting are increasing year by year, which can be clearly seen in Fig. 2. Moreover, this technology is quite suitable and advantageous for non-electroactive molecule detection. Non-electroactive species are molecules that cannot be transformed by electrochemical reactions such as pesticides, drugs, etc. Therefore, they can be measured by affinity techniques, or catalytic secondary molecule usage. Secondary molecule usage, however, has disadvantages such as secondary molecule and target molecule interaction, solvent problems, where template and secondary molecule may not be solved in the same solvent or harsh
conditions can affect the reaction of target molecule, hence the measurement. Therefore, affinity measurement is very beneficial for these kinds of molecules. Affinity measurement is used to detect molecules depending on the affinity between target molecule and the molecule it shows affinity. In biosensor technology DNA, antibody, protein, and receptor-based systems are designed which could be collectively called affinity-based systems. However, these biocompounds are expensive, hard to immobilize onto transducers, and challenging to study on their optimum conditions. Then, an idea came up to the scientists to avoid these disabilities for use of MIPs on transducer surfaces. MIP-based sensors have been constructed since then as electrochemically, optically, and piezoelectrically.

![Figure 2](image.png)

**Figure 2.** The number of papers referring to biosensor based on MIPs in the last 15 years (searching was performed using “molecularly imprinted sensors” as search key terms on Google Scholar [29]).

### 4.1. Electrochemical MISens

The fundamentals of electrochemistry are to study the interaction between matter and electricity (Fig. 3). This interaction gives information and provides quantitative measurement of the analyte. Electrochemical techniques of MISens mostly measure surface properties of the transducers, binding kinetics and polymer rearrangements. In this section we gave examples of electrochemical MISens.

Silva and co-workers designed a novel electrochemical sensor for the determination of trimethoprim by electropolymerization of pyrrole (PY) and molecularly imprinted polymer (MIP) which was synthesized onto a glassy carbon electrode (GCE) in aqueous solution using cyclic voltammetry. In their study, they used graphene (GNPs) in order to enhance the sensitivity of the sensor by an increase in the electrochemical conductivity. The performance of the imprinted and non-imprinted (NIP) films was investigated by electrochemical impedance spectroscopy (EIS) and the cyclic voltammetry (CV) of a ferric solution. The sensor they
developed presented a linear range between peak current intensity and logarithm of TMP (trimethoprim) concentration with a range from $10^{-6}$ to $10^{-4}$ M. The results were accurate (with recoveries higher than 94%), precise (with standard deviations less than 5%), and the detection limit was $1.3 \times 10^{-7}$ M [30].

Xue et al. reported an electrochemical sensor for the amperometric detection of dopamine that was carried out via gold nanoparticles doped MIP. In this work, dopamine (DA) was used as the template molecule, functionalized AuNPs (F-AuNPs) as functional monomers and p-aminobenzenethiol (p-ATP) as the cross-linker. They synthesized MIP following these steps: An electrolyte solution containing 1 mmol L$^{-1}$ DA, 10 mmol L$^{-1}$ F-AuNPs, 7 mmol L$^{-1}$ p-ATP, and 0.1 mol L$^{-1}$ ABS (acetate buffer solution) (pH 5.0) was kept in the dark under a nitrogenous atmosphere at room temperature for 6 h to complete the pre-assembly between DA and F-AuNPs through the hydrogen-bond interaction. The AuNPs-modified electrode was immersed into the electrolyte solution and the AuNPs@MIES (gold nanoparticle and MIPs modified sensor) was prepared by the electropolymerization at a constant potential of 1.0 V for 400 s. After that, the electrode was immersed in 0.5 mol L$^{-1}$ H$_2$SO$_4$ and treated with a constant potential of $-0.5$ V for 400 s to remove the templates and dried under nitrogen flow. The developed sensor effectively minimized the interferences caused by ascorbic acid (AA) and uric acid (UA). Also according to linear range (0.02 μmol L$^{-1}$ to 0.54 μmol L$^{-1}$) and detection limit (with the detection limit of 7.8 nmol L$^{-1}$) of reported dopamine sensor, it can be said that the developed sensor exhibited high sensitivity and high selectivity [31].

Yu et al. designed a molecularly imprinted electrochemical sensor based on nickel nanoparticle-modified electrodes for phenobarbital determination. Reported electrochemical sensor was developed by thermal polymerization with the use of methacrylic acid (MAA) as the functional monomer, 2,2-azobisisobutyronitrile (AIBN) and ethylene glycol maleic rosinate (EGMRA)
acrylate as the crosslinking agent, phenobarbitals (PBs) as the template molecule, and dimethyl sulfoxide (DMSO) as an organic solvent. In the sensor fabrication process, 0.0464 g PB and 0.0688 g MAA were mixed in 3 mL DMSO and sonicated for 10 min. After 5 h, 1.0244 g EGMRA and 0.0074 g AIBN were added into the mixture and sonicated for 30 min to obtain PB-imprinted polymer solutions. After that, 10 μL of 2.0 mg mL$^{-1}$ Ni nanoparticle solution dropped on the GCE surface and then the sensor was dried at room temperature. Approximately 5 μL of the prepared PB-imprinted polymer solution was then coated on the Ni nanoparticle-modified GCE and vacuum dried at 75°C for 6 h. Following the thermal polymerization, the imprinted sensor was washed with (acetic acid) HAc/methanol (volume ratio, 3:7) for 7 min to remove the template molecules. The electrochemical properties of the modified molecularly imprinted and non-imprinted polymer sensors were investigated by cyclic voltammetry, differential pulse voltammetry, electrochemical impedance spectroscopy, and chronocoulometry. Under optimized conditions, the currents were found to be proportional to the PB concentrations within a range of $1.4 \times 10^{-7}$ mol L$^{-1}$ to $1.3 \times 10^{-4}$ mol L$^{-1}$ ($r^2 = 0.9976$), with a detection limit of $8.2 \times 10^{-9}$ mol L$^{-1}$. The developed sensor was used to determine PB in actual fish samples [32].

Anirudhan and co-workers reported molecularly imprinted polymer-based potentiometric sensor from the surface modified multiwalled carbon nanotube (MWCNT) for the determination of an organochlorine pesticide that is lindane ($\gamma$-hexachlorocyclohexane). A MWCNT modified imprinted electrochemical sensor was developed by the following these steps: MWCNT-CH=CH$_2$ was added to the solvent mixture of 60 mL of acetonitrile and 10 mL of toluene in a 500 mL round-bottom flask. After that the mixture was purged with N$_2$ gas under a constant magnetic stirring. A mixture of $\gamma$-HCCH ($\gamma$-hexachlorocyclohexane) and MAA was prepared and dissolved in 35 mL of N,N-dimethylformamide. It was stirred for 30 min to get a compound of template molecule and functional monomer. To that mixture, the cross linker ethylene glycol dimethacrylate (EGDMA) and initiator AIBN were also added; the reaction was allowed to proceed for 16 h at 70°C. Ethanol was used to remove template molecules. A MWCNT was grafted using glycidyl methacrylate (GMA). The reaction of MWCNT with GMA produces MWCNT-g-GMA and the epoxide ring present in the GMA upon reaction with allylamine produces the vinylated MWCNT (MWCNT-CH=CH$_2$). MWCNT-based imprinted polymer (MWCNT-MIP) was synthesized by means of methacrylic acid (MAA) as the monomer, EGDMA as the cross linker, $\alpha,\alpha'$ azobisisobutyronitrile (AIBN) as the initiator, and $\gamma$-HCCH, an organochlorine pesticide molecule, as the template. The properties of the modified molecularly imprinted and non-imprinted polymer sensors were investigated by linear sweep voltamgrams, FTIR, XRD, Raman spectra, and TEM analyses. This developed sensor presented a linear range of $10^{-10}$–$10^{-3}$ M and the detection limit of $10^{-10}$ M [33].

Patra and co-workers developed a molecular imprinting-based sensor for medullary thyroid carcinoma marker. The fabrication of the sensor was made by the following steps. Accordingly, bipyridyl (0.2 mmol) and CuCl$_2$ (0.1 mmol) were dissolved in 2 mL DMSO (dimethyl sulfoxide) to obtain a solution of Cu(II)-complex. Subsequently, this complex was mixed with a ZnO nanostructure modified monomer (10 mg, 1.0 mL DMSO), template (calcitonin, 2.0 mg, 1.0 mL DMSO), and EGDMA (ethylene glycol dimethacrylate) (1 mmol, 180mL) in the presence of
ascorbic acid (0.1 mmol) as the reducing agent. A sharp colour change from light blue to green indicated the in situ reduction of Cu(II)-complex to Cu(I)-complex that catalysed the chain propagation in the presence of the ethyl-2-bromo isobutyrate (2 mmol, 300mL) as initiator. The whole mixture was purged with N2 gas for 10 min. A drop of this mixture (5.0μL) was spread over the protruding tip of the functionalized PGE and kept in a pre-heated oven for half an hour at 45°C, resulting in calcitonin adduct polymer modified electrochemical sensor. The morphologies and properties of the developed sensor were characterized by scanning electron microscopy, cyclic voltammetry, difference pulse voltammetry, and chronocoulometry. Linear responses of the imprinted sensor to calcitonin were observed for concentrations ranging from 9.99 ng L\(^{-1}\) to 7.919 mg L\(^{-1}\) and the detection limit was as low as 3.09± 0.01 ng L\(^{-1}\). The reported imprinted electrochemical sensor was used to determine the concentration of calcitonin in the human blood serum samples [34].

Karimian and co-workers reported an on/off-switchable molecularly imprinted polymer (MIP) affinity sensor for folic acid using copolymerization of poly(N-isopropylacrylamide) (PNIPAAm) with a cross-linker (N,N’-methylenebisacrylamide) (MBA) and additional monomer (o-phenylenediamine (o-PD)), in the presence of folic acid as template. Polymerization was carried out following these steps: The folic acid molecularly imprinted film was prepared by the electrochemical polymerization of PNIPAAm and o-PD on the surface of gold electrode, using cyclic voltammetry in the potential range between 0 and 1.1 V (versus Ag/AgCl), for 20 cycles at a scanning rate of 50 mV s\(^{-1}\). The polymerization mixture consisted of an aqueous solution containing 10 mM o-PD, 2.5 mM PNIPAAm, 2.5 mM MBA, and 0.2 mM folic acid. For the preparation of the polymers, the components were dissolved in acetate buffer (0.5 M, pH 5.8). For the washing procedure, the polymer film was rinsed in methanol-acetic acid (9:1, v/v) solution for 20 min at 50°C, followed by subsequent washing with methanol to remove the template entrapped in the polymeric matrix. The electrochemical behaviour of the thin film (MIP) was characterized using differential pulse voltammetry and cyclic voltammetry. Reported sensor response shows a limit of detection of 0.9 μM with linear range from 1.0 μM to 200 μM [35].

Wang et al. have developed an electrochemical sensor for the determination of aflatoxin B\(_1\) based on MWCNT-supported Au/Pt bimetallic nanoparticles. This study involves a molecularly imprinted sensor technology, which was a modification of glassy carbon electrode (GCE) by o-phenylenediamine (OPD), electrochemically. Carbon nanotubes were used as support material and supported by Au/Pt bimetallic nanoparticles. Moreover, this layer formation was monitored by cyclic voltammetry (CV). Amine groups on OPD were the donor of hydrogen to form hydrogen bonds between AFB1’s oxygen. After MWCNT coating, Au/PtNPs were deposited onto MWCNTs-GCE. DP and CV measurements were carried out by using Fe(CN)\(_6\) redox solution. Template molecule was removed by using HCl solution pH=2 for 9 min. A linear relationship between the sensor response signal and the logarithm of AFB1 concentrations ranging from 1×10\(^{-10}\) to 1×10\(^{-5}\) mol L\(^{-1}\) was obtained with a detection limit of 30 pikomol L\(^{-1}\). It was applied to detect AFB1 in hogwash oil successfully [1]. As you can understand, the main objective of this study is based on examination of the surface characteristics of the modified electrode. Ferricyanide oxidation/reduction peaks altered, when selective cavities of OPD/MWCNT-Au/Pt layer bind the AFB1 [36].
Uygun and Dilgin developed a novel impedimetric sensor based on molecularly imprinted polypyrrole (PPy) modified pencil graphite electrode (PGE) for trace level determination of chlorpyrifos (CPF), which is a pesticide. In this study, they used PGE as transducer, and PGE was modified by pyrrole electrochemically formed polymers on electrode by cyclic voltammetry, and CPF was used in polymerization process simultaneously. CPF was used as template and removed after polymerization by using pH=2 HCl solution to remove H bonds between PPy and CPF. The whole surface polymerization steps and measurement steps were examined by electrochemical impedance spectroscopy, which is an electrochemical electron resistance-based surface characterization technique, by using ferri/ferrocyanide redox probes. Under experimental conditions, the proposed impedimetric sensor has a linear response range from 20 to 300 μg L\(^{-1}\) CPF with a detection limit of 4.5 μg L\(^{-1}\) (based on 3σb). Furthermore, the fabricated sensor was successfully applied to determine CPF in CPF-added artificial corn leaves, tap water, and soil samples. Two types of organophosphates and two metabolite of CPF that chlorpyrifos oxon (CPFO) and 3,5,6-trichloro-2-pyridinol (TPD) and 2,4-dichlorophenoxyacetic acid (2,4D) which is a common systemic pesticide/herbicide were selected for the control experiments [13].

Zhong et al. have developed a pyrrole–phenyl boronic acid: a novel monomer for dopamine (DA) recognition and detection based on imprinted electrochemical sensor. They used a new monomer for MIP by synthesizing pyrrole-phenyl boronic acid. In this study GCE used as a transducer. Dopamine was used as template and polymerization was performed by CV. DA was extracted by H\(_2\)SO\(_4\) and applied electrical force 0–0.15 V to remove DA from imprinted polymer cavities. Differential pulse voltammetry (DPV) was used as measurement method, a linear ranging from 5.0x10\(^{-8}\) to 1.0x10\(^{-5}\) mol L\(^{-1}\) for the detection of DA was obtained with a detection limit of 3.3 × 10\(^{-8}\) mol L\(^{-1}\) (S/N=3). For the recovery tests, the samples were spiked with 4.0 × 10\(^{-6}\) mol L\(^{-1}\), 6.0 × 10\(^{-6}\) mol L\(^{-1}\), and 8.0 × 10\(^{-6}\) mol L\(^{-1}\) DA varied from 91.5% to 105.2% [37].

For another study of electrochemical sensor, Wang et al. developed a sensor technology that is an ultrasensitive molecularly imprinted electrochemical sensor based on magnetic graphene oxide/β-cyclodextrin (CD)/Au nanoparticle composites for chrysoidine, which is an azoic dye. As you can read, a magnetic graphene oxide (MGO), cyclodextrin, which has hydrophobic and hydrophilic residues, and gold nanoparticles as electrical conductive material were used. GCE was used as the transducer for measurements. MGO/CD@AuNPs modified GCE was put in a solution, which contains pyrrole and chrysoidine together to form an imprinted material by employing CV method. After polymerization, the template molecule was removed from the surface by soaking modified GCE in ethanol. The surface of both non-imprinted and imprinted sensor system was characterized by SEM (scanning electron microscopy), EIS, and CV measurements. The measurement system was based on the differential pulse voltammetry (DPV) to quantify chrysoidine. The calibration curve data was between 5.0 × 10\(^{-6}\) and 5.0 × 10\(^{-3}\)mol L\(^{-1}\). The detection limit was estimated to be 1.7 × 10\(^{-6}\) mol L\(^{-1}\) at a signal-to-noise ratio of 3σ (where σ is the standard deviation of the blank, n = 6) [38].

Yola et al. reported a study where a molecularly imprinted electrochemical biosensor based on Fe@Au nanoparticles involved in 2-amino ethanethiol (2-AET) functionalized multiwalled
carbon nanotubes was developed for the sensitive determination of cefixime (CEF) in human plasma. In this study, they modified a GCE by p-nitro phenyl diazonium tetra fluoro borate (p-NPDEFB) salt in MeCN with TBATFB (Tetrabutylammonium tetrafluoroborate) using CV, reduced the formed nitro groups by applying negative voltage, activated MWCNT tubes that were attached onto the modified electrode surface, and 2-AET and Fe@Au layers were formed by self-assembling, respectively. After electrode surface modification, the modified electrode was soaked in a solution, which contains pyrrole and CEF, to form CEF imprinted layers by using CV. NaCl solution was used as a desorption agent of CEF. For the measurement, square wave voltammograms were used as a function of concentration. Limit of detection (LOD) was calculated as $2.2 \times 10^{-11}$ M and the calibration curve was created from 0.1 nM to 10nM [39]. Oxygen groups on the CEF and N groups on the PPy were the fundamentals of attraction of specified cavities.

4.2. Surface plasmon resonance MISens

Surface plasmons are formed by an electromagnetic wave, which propagate along the surface of a thin metal layer. According to Abbas et al., surface plasmon resonance (SPR) is a collective oscillation of conduction electrons, which present at the interface of metal-dielectric media. SPR have three features, important in terms of any new sensor: firstly the enhancement of the electric field, secondly the propagation length, and the lastly the penetration depth [40].

The SPR phenomenon was recognized in 1960s after Otto and Kretschmann had invented surface plasma with invisible light. The SPR sensor technique has been used in very different areas for immunosensors, the determination of interaction between immunoglobulin G (IgG) protein and antigen, monitoring of the interactions between drugs and biological molecules, and so on [41]. There are plenty of planar configurations of SPR biosensors. Among these, in general, Otto configuration is used. Generally, SPR sensor is formed of six parts, including a light source, a detector, a transduction surface, a prism, biomolecule, and a flow system.

A typical SPR system (immunoassay technique is described), as mentioned above and can be seen in Fig. 4., uses microfluids to pass controlled amounts of analyte across the sensor surface to which the antibody is immobilized. With reflecting a beam of polarized light to the back surface of the metal film, the analysis is made through a prism. After the beam of light hits the noble metal surface, not all the light is reflected. Some of the energy of photons is absorbed by the metal and causes electron oscillations at the interface of two materials. When molecules are bound to the sensor surface, the refractive index (RI) changes. RI affects also reflected light intensity, angle, and wavelength. It is measured as resonance units (RU). In general, 1 RU is equal to 1 pg mm-2 of analyte concentration [41].

As can be seen from Fig. 4, there are two mediums and an interface. One medium is optically denser. When light passing from the optically denser medium is exposed to the light-thinning medium, at the interface of two mediums, total reflection will occur, if an appropriate range of incident angles are inherent in the medium, change of resonance amplitude occurs, with a penetration depth. For example, if antibody was hold on sensor chip and was let to interact with antigen solution, the refractive index (RI) of the metal film surface would change. The change of SPR resonance angle would change with a change of refractive index. The change
of refractive index will be proportional to mass change, due to the absorption of antigens to antibodies. This means that the mass change of biological macromolecules causes refractive index change with SPR resonance angle change [42].

Optical sensor research has very advantageous features that it allows label-free analysis, it is simple to construct, and has the ease of use – inexpensive and highly sensitive [41].

Carlucci et al. made a study to determine Vitamin D (25OHD) with a novel optical and electrochemical-based biosensor. For SPR measurements, first, gold SPR disks were cleaned with fresh piranha solution (3:1 H\(_2\)SO\(_4\), 98% : H\(_2\)O\(_2\), 30%). Then, a self-assembled monolayer with 11-mercaptoundecanoic acid (MUA) was formed on gold surface. The carboxyl functions on the SAM layer were activated with a mixture of N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS). After removing the mixture, making later steps, a LOD of 2 \(\mu\)g mL\(^{-1}\) but when vitamin D was modified with gold nanoparticles (AuNPs) a lower LOD of 1 \(\mu\)g mL\(^{-1}\) was reached. With an electrochemical biosensor, which was based on the reaction of vitamin D with 4-ferrocenylmethyl-1,2,4-triazoline-3,5-dione (FMTAD), vitamin D was determined with a LOD of 10 ng mL\(^{-1}\) [44].

In a study of Choi et al., Zearalenone, found in a number of cereal crops, was determined via surface plasmon resonance sensor. Zearalenone is a mycoestrogen, which acts like an endocrine disruptor. For determination, pyrrole was electropolymerized in the presence of Zearalenone. Electropolymerization was made with a three-electrode electrochemical system. Au film was used as the working electrode, Ag/AgCl as reference, and Pt grid as counter. After that, PPy-coated Au chips were mounted on the SPR cell and change of incident angle of laser was measured. According to the results, the sensor exhibited a linear response in the range of 0.3–3000 ng mL\(^{-1}\) with a LOD of 0.3 ng g\(^{-1}\). For selectivity test, structural analogues of Zearalenone were used (\(\alpha\)-Zearalenone, Zearale-
none, β-Zearalenone). Among these compounds, the sensor showed the highest selectivity to Zearalenone, due to the strong binding capacity [45].

Yao et al. made a SPR sensor to determine pesticide, which has high toxicity and binds irreversibly to acetylcholinesterase (AChE). Because of this, it causes serious harm in the respiratory tract, human nervous system, and cardiovascular system. To detect and enhance detection sensitivity, magnetic molecularly imprinted nanoparticles (NPs) were used. Magnetic NPs were prepared through the self-polymerization of dopamine on the Fe₃O₄ NP surface in the presence of template, chlorpyrifos (CPF). Using these NPs, pesticide was detected in a range from 0.001 to 10 μM with a detection limit of 0.76 nM [46].

Like other examples, the SPR technique is also used for the detection of another organic molecule, domoic acid (DA), which is a neurotoxic amino acid. This toxin accumulates in mussels (such as Mytilus edulis), crabs or anchovies and when DA-contaminated shellfish is taken, called an intoxication syndrome known as amnesic shellfish poisoning (ASP) can occur. The typical symptoms of ASP are vomiting, cramps, and diarrhoea and neurological symptoms including severe headache, seizures, and either temporary or permanent memory loss. Lotierzo et al. prepared a MIP film by direct photo-grafting onto a gold chip. Firstly, the gold surface was functionalized with a self-assembled monolayer of 2-mercaptoethylamine and subsequent carbodiimide. This provided covalent attachment of the photo initiator 4,4’-azobis(cyanovaleric acid). After proper steps, DA containing polymerization solution was deposited on the gold surface, and for polymerization the chip was irradiated with ultraviolet light. Non-printed control chips were prepared with the same procedure but without the template, DA. According to the results, DA was detected in a range of 2-3300 μg L⁻¹. After a number of tests, molecularly imprinted DA sensor protected its stability until 30 (±5) analysis [47].

Molecular imprinting based surface plasmon resonance technology can be applied also for enzyme detection. Matsunaga et al. prepared molecularly imprinted polymers for lysozyme with acrylic acid (AAc) as the functional monomer, and N,N’-methylenebisacrylamide (MBAA) as the cross-linker. For preparation of SPR sensor, firstly an Au-coated SPR sensor chip was immersed in N,N’-bis(acryloyl)cystamine to bear vinyl groups on Au surface. A polymerization mixture (including template, lysozyme) was applied on Au and polymerized by radical polymerization. For polymerization, the vinyl group grafted SPR sensor chip was poured on glass, on which the polymerization mixture had been poured. After that another glass plate and a weight were placed on the sensor. A non-imprinted polymer thin film was prepared with the same procedure but without adding template. After proper steps, SPR measurements were made. To examine the effect of salt concentration on the rebinding of lysozyme, imprinted sensors were prepared in various concentrations of NaCl (0, 20, 40 mM). It was seen that the bound amounts of proteins were decreased with the increasing concentrations of NaCl in the rebinding buffer. From this result, it can be thought that electrostatic interactions took place between proteins and acrylic acid residues. With examples of different proteins, it was seen that binding changed upon isoelectric points of amino acid residues. For example at pH 7.4, lysozyme (pI:11), Cytochrome C (pI:10), and RNAse (ribonucleotidase) (pI: 9.5) were positively charged and strongly bound to the films via acrylic acid residues. At the
same pH value, myoglobin (pI:7) and lactalbumin (pI:4.5) were negatively charged and that is why they showed almost no binding because of this electrostatic repulsion. This study is also an example for selective protein sensors with SPR sensing technique [48].

Enterotoxins can be detected with SPR technique just in the same way as with quartz crystal microbalance (QCM) sensors. Homola et al. developed a new SPR sensor for *Staphylococcal enterotoxin B* (SEB), which is a soluble protein, secreted by *Staphylococcus aureus*. According to the results SEB could be detected at low concentrations, such as 5.0 μg L⁻¹, in pure samples, directly. But, by using a sandwich assay, this limit has been decreased to 0.5 μg L⁻¹ in both pure samples and in milk [49].

Food allergens can be detected via SPR technology. Yman et al. detected peanut allergen protein with optical sensor with both direct and sandwich immunoassays. By these methods they detected milk, hazelnut, sesame, egg, and peanut proteins in food samples. They used polyclonal antibodies to detect these allergens. According to the results, allergens were detected in the range of 1.0–12.5 μg g⁻¹ in food samples [50].

As it can be seen from these examples, SPR sensing technology can be used in a variety of areas changing from protein detection to environmental pollutants with a low detection range, faster attainment of results, and selectivity.

### 4.3. Quartz Crystal Microbalance (QCM) MISens

The QCM consists of a thin piezoelectric plate, which has acoustic resonances in the MHz range. When the crystal comes into contact with the sample, the resonance properties change. QCM technology was first recognized by Sauerbrey in 1959. He indicated usefulness of the method for measuring the characteristic frequency of an oscillator circuit. The frequency changes were determined by using a piezoelectric crystal and as can be seen in Fig. 5, the oscillating frequency of the crystal decreases with the adsorption of foreign substances on the surface.

*Figure 5.* Frequency change on QCM electrode, while interacting with sample.
Because of the sensitive nature of quartz crystal, this method was described as a very precise method. The results of this work are embodied in the equation. According to the Sauerbrey equation, the mass change per unit area at the QCM electrode surface and frequency changes are proportional.

The observed change in oscillation frequency of the crystal:

\[ \Delta f = -C_f \Delta m \]  

where

\( \Delta f = \) the observed frequency change (Hz)

\( \Delta m = \) the change in mass per unit area (g/cm\(^2\))

\( C_f = \) the sensitivity factor for the crystal (56.6 Hz μg\(^{-1}\) cm\(^2\) for a 5 MHz AT-cut quartz crystal at room temperature)

As mentioned above, the Sauerbrey equation relies on a linear sensitivity factor, \( C_f \), which is a fundamental property of the QCM crystal. The method was also utilized for the direct weighed of a mass [51]. QCM, which is used for the biosensor experiment, is consisted of piezoelectric crystal, oscillator, and frequency counter(FIG 6.).

The piezoelectric quartz crystal is driven by a low-frequency transistor oscillator. The frequency of the vibrating crystal is monitored by the frequency counter. The crystal, which is mounted on its holder, is connected to the oscillator circuit. The frequency counter is connected to the oscillatory device. By frequency counter, frequency changes are recorded after each step in coating or in interaction with the sample.

**Figure 6.** The schematic diagram of experimental piezoelectric sensor.
The advantages of the technique are surface specificity, monolayer sensitivity, and high acoustic contrast for dilute adsorbents [52]. Besides QCM does not require any labelling, has low barriers of entry, ease-of-use, low cost, and speed to result [54].

The theoretical detection limit of oscillating quartz crystals is about 10–12 g, which means a detection in pictogram range. With this low detection limit, the QCM can be used in trace analysis, immunosensors, DNA biosensors, and drug analysis. Piezoelectric crystals can also be used in microbalances for thin film technology [53].

Because of expense, sensitivity, and short shelf life of biological materials, molecularly imprinted polymers (MIPs) are used on sensor surface. In addition in QCM analysis, MIPs are used to achieve a specific binding site and a high affinity. MIPs are advantageous because of their features, like high similarity to natural receptors, physicochemical, mechanic, thermal stability, simple preparation, and easy adaptation of application [53].

The crystal frequency changes when the interaction occurs between imprinted polymer and template solution. It can be seen that before and after the interaction crystal frequency decreases due to the uptake of template by the imprinted polymer. Liao et al. made a study for stereospecific L-histidine sensor with imprinted polyacrylamide membranes. According to the study when the crystals were interacted with L-histidine, the net frequency shifts of the crystal modified with L-histidine is found much more than the shifts, which belongs to D-histidine. It can be concluded that the L-histidine imprinted membrane showed better selectivity to L-histidine. Besides specificity, selectivity is also an important feature of imprinted polymers. In this study, L-tyrosine and L-arginine were tested with the L-histidine imprinted membrane and DL-phenylalanine was tested with D-histidine imprinted membrane. Under the same reaction conditions (time, concentration, etc.), the imprinted membrane showed much more affinity to the same molecule, which was used as template because of specific cavities, formed in the polymer. From these results, it can be concluded that this imprinted piezoelectric sensor can be used for the chiral separation of histidine [55].

In another study, Liu et al. reported a novel method for the separation of D- and L-tryptophan using molecularly imprinted quartz crystal microbalance (QCM) sensor. They fabricated the sensor by using molecularly imprinted polymers, which was prepared by using acrylamide (AM) as monomer and 1,1,1-trimethylolpropane trimethacrylate (TRIM) as cross-linker in different molar ratios. With the fabricated optimum imprinted polymer, the binding of template L-tryptophan was about four-fold to three-fold larger than that obtained with the D-tryptophan enantiomer. It was calculated that the enantiomeric selectivity coefficient of the fabricated molecularly imprinted sensor was 6.4. Moreover, it was observed that the binding of L- and D-tryptophan enantiomers on the non-imprinted polymer (NIP) was almost the same. This indicated the sensitivity and enantioselectivity of molecularly imprinted polymer [52].

It is also possible to make mass determinations in protein mixtures via MIP-QCM sensor. Lin et al. prepared an albumin imprinted copolymer of 3-dimethylaminopropyl methacrylamide (DMAPMA) and different acrylate series cross-linking agents. Gold surface was used and four kinds of Au-coated crystals were prepared. One of them was bare and the other three were prepared with different functional groups bonded to the surface of the sensor. As functional
groups, they used –NH₂, –OH, –COOH on Au surface. According to the results, the greatest adsorption capacity belonged to Au-OH electrode and to a lesser extent, to bare Au electrode. However, according to the time effectiveness to obtain stability and according to the adsorbed albumin amount, Au-NH₂ and Au-OH were the optimal electrodes. Between NIP and MIP electrodes prepared, the molecularly imprinted electrode showed more efficient albumin determination than non-imprinted electrode with the time taken to receive a steady state frequency and adsorbed amount of albumin. In addition to this, the prepared albumin-imprinted QCM sensor showed largest adsorption of albumin among similar molecules like cytochrome c, lysozyme, and myoglobin, whose molecular sizes were far smaller than albumin. In the range between 60 and 150 ppm, albumin was obtained. With results of this study, it can be indicated that the presence of albumin-specific cavities in the prepared electrode gave a greater adsorption and a smaller diffusion resistance, which makes response time shorter [53].

Sun et al. used piezoelectric quartz crystal for sensing taste-causing molecules by using molecular imprinting technology. A PQC (piezoelectric quartz crystal) sensor array, which is MIP coated, is developed to quickly and more sensitively detect taste-causing compounds in beverage. They studied quinine, which is a bitter-taste causing compound, and usually flavoured with saccharine to reduce its unpleasant bitter taste. Because of this they used quinine and saccharine as template molecules. Methacrylic acid (MAA) is used as a monomer. The MIP coated PQC sensor array was studied under flow injection analysis and results were compared with the results of volunteer human taste panellists. With the satisfactory repeatability, and with a high sensitivity to detect the change in bitter taste in tonic water with much less suppressing effect in the presence of saccharine, the developed sensor was very comprehensive. According to results, the quinine-MIP modified PQC sensor displayed a linear working range for quinine from 10 mg L⁻¹ to 1080 mg L⁻¹ and for saccharine from 51 mg L⁻¹ to 3420 mg L⁻¹. The calculated limit of detection is 2.04 mg/L for quinine and 32.8 mg L⁻¹ for saccharine [56].

MIP-PQC is used not only for taste application but also to distinguish different taste causing compounds [57] and to detect organic pollutants with taste implication such as organic/inorganic acids and amines in drinking water [58].

Due to the use of QCM in the gas phase, it has application as odour sensors. Ji et al. used 2-methylisoborneol (MIB) and geosmin (GEO) as off-flavour compounds which cause odour problems in drinking water. They are produced by some microorganisms. These odour chemicals were analysed with GC-MS or Enzyme-Linked Immunosorbent Assay (ELISA) and detection limits were ca. 1 ng/L and 1 μg/L, respectively. But these methods need a high budget in terms of chemicals and equipment. But with the piezoelectric sensor, the analysis could be done at a lower cost and more sensitively. In their study, they made pre-treatment with nylon layers to QCM electrode. After that, they used MIB and GEO as template molecules and imprinted polymers are prepared with methacrylic acid as functional monomer, ethylene glycol dimethacrylate as cross-linker, and 2,2’-azobis (2,4-dimethyl)valeronitrile as initiator. They were all dissolved in hexane, used as porogen, under nitrogenous atmosphere. Five microliters of this solution were pipetted onto prepared QCM and polymerized at 40°C for 48
Non-imprinted polymers were synthesized under the same conditions except the use of the template. The prepared QCMs were interacted with template molecules in a thermostat chamber in a stream of nitrogen flowing. After interaction, imprinted sensors showed an average frequency change of 2864 Hz ± 6.26% (n=3) and NIP-sensors 3014 Hz ± 5.14%. It indicates a similar amount of substrate immobilization on sensors (ca. 3 μg). It was observed that the frequency change after MIP application to the nylon sublayer was about 50% higher than after application of the MIP to a bare QCM. To analyse selectivity except MIB and GEO, some other odorants like terpinol, β-ionone, and citronellol were also interacted with MIB-imprinted sensor and it was found that the highest frequency change was observed at MIB-sensor after interaction with MIB. In spite of their previous sensor with an LOD of 200 ppb, these synthesized sensors could detect above 10 ppb. This means approximately 20-fold more sensitive detection capacity [59].

QCM sensor could be used for influenza detection. Either influenza virus can be detected or influenza virus binding capabilities can be analysed. Diltemiz et al. have developed a sensor for recognition of the hemagglutinin (HA) protein, which occurs by influenza virus with infection and causes hemagglutination. For this, they used 4-aminophenyl boronic acid (4-APBA) as a new ligand for binding of sialic acid (SA), which has a valuable role in the binding of HA through boronic acid sugar interaction. QCM sensor surface was modified with thiol groups and then 4-aminophenyl boronic acid and sialic acid were immobilized on sensor surfaces, respectively. To do these, first QCM electrodes were cleaned with alkaline piranha solution (1:1:5 deionized water : H₂O₂ : NH₃ v/v). After cleaning, electrode surfaces were modified with 11-mercaptopoundecanoic (MUA) acid. By using MUA, carboxyl fictionalization were achieved. After that, QCM electrodes were modified with imide groups by using N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide and N-hydroxysuccinimide for immobilization of 4-APBA and SA. After interaction with the samples, the binding capacity and limit of detection of QCM sensors were found to be 4.7×10⁻² μM and 0.26 μM ml⁻¹, respectively [60].

As mentioned above, QCM finds lots of application areas in terms of low cost, speed to result, and low detection limit. Because of these advantages, studies made with QCM sensor are increasing day by day.

5. Comparison of MI-sensors

In this section we compared MISens by showing Table 1 to describe polymer type, measurement type, LOD, and detection range.

<table>
<thead>
<tr>
<th>Sensor Type</th>
<th>Modification</th>
<th>Target Molecule</th>
<th>Detection Range</th>
<th>LOD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impedimetric</td>
<td>PGE/PPy</td>
<td>CPF</td>
<td>20 to 300 μg L⁻¹</td>
<td>4.5 μg L⁻¹</td>
<td>13</td>
</tr>
<tr>
<td>Impedimetric/voltammetric</td>
<td>GCE/PPy</td>
<td>Trimethoprim</td>
<td>10⁻⁴–10⁻⁸ M</td>
<td>0.13 μM</td>
<td>30</td>
</tr>
<tr>
<td>Sensor Type</td>
<td>Modification</td>
<td>Target Molecule</td>
<td>Detection Range</td>
<td>LOD</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------</td>
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<td>-----------------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>Amperometric</strong></td>
<td>AuE/ AuNP@MIES</td>
<td>Dopamine</td>
<td>7.8 nM</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td><strong>CV/DPV/ Amperometric</strong></td>
<td>GCE/Ni/MIP</td>
<td>Phenobarbital</td>
<td>0.14 μM–1.3 mM</td>
<td>8.2 nM</td>
<td>32</td>
</tr>
<tr>
<td><strong>Potentiometric detection</strong></td>
<td>CuE/MWCNT- MIP</td>
<td>γ- hexachlorocycloexane</td>
<td>$10^{-5}$–$10^{-3}$ M</td>
<td>$10^{-10}$ M</td>
<td>33</td>
</tr>
<tr>
<td><strong>CV/DPV/ chronocoulometry</strong></td>
<td>PGE/MAA</td>
<td>Calcitonin</td>
<td>9.99 ng L$^{-1}$–7.919 mg L$^{-1}$</td>
<td>3.09 ng L$^{-1}$</td>
<td>34</td>
</tr>
<tr>
<td><strong>DPV/CV</strong></td>
<td>AuE/ PNPAAm/o-PD</td>
<td>Folic Acid</td>
<td>1–200 μM</td>
<td>0.9 μM</td>
<td>35</td>
</tr>
<tr>
<td><strong>DPV/CV</strong></td>
<td>GCE/ MWCNT/Au-Pt/oPD</td>
<td>Aflatoxin B1</td>
<td>$1\times10^{-10}$–$1\times10^{-5}$ mol L$^{-1}$</td>
<td>30 pmol L$^{-1}$</td>
<td>36</td>
</tr>
<tr>
<td><strong>DPV</strong></td>
<td>GCE/ AuNPs/oCD/MGO</td>
<td>Chrysodine</td>
<td>$5.0 \times 10^{-4}$ – $5.0 \times 10^{-4}$ mol L$^{-1}$</td>
<td>$1.7 \times 10^{-4}$ mol L$^{-1}$</td>
<td>38</td>
</tr>
<tr>
<td><strong>SWV</strong></td>
<td>GCE/MWCNT/p- Cefexime</td>
<td>Cefexime</td>
<td>$0.1$ nM–10 nm</td>
<td>$2.2 \times 10^{-10}$ M</td>
<td>39</td>
</tr>
</tbody>
</table>

**Table 1.** Modification, measurement type, LOD, and detection range

<table>
<thead>
<tr>
<th>Sensor Type</th>
<th>Modification</th>
<th>Target Molecule</th>
<th>Detection Range</th>
<th>LOD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optical</strong></td>
<td>GOx/PtOEP</td>
<td>Glucose</td>
<td>2–120 mg dL$^{-1}$</td>
<td>$1.5\pm0.2$ mg dL$^{-1}$</td>
<td>44</td>
</tr>
<tr>
<td><strong>Optical</strong></td>
<td>Au/MUA</td>
<td>Vitamin D</td>
<td>0.05–1.0 μg mL$^{-1}$</td>
<td>0.045 μg mL$^{-1}$</td>
<td>45</td>
</tr>
<tr>
<td><strong>Optical</strong></td>
<td>Au/Pyrrole</td>
<td>Zearalenone</td>
<td>0.3–3000 ng mL$^{-1}$</td>
<td>0.3 ng g$^{-1}$</td>
<td>46</td>
</tr>
<tr>
<td><strong>Optical</strong></td>
<td>Au/MUA</td>
<td>Chlorpyrifos (CPF)</td>
<td>0.001–10 μM</td>
<td>0.76 nM</td>
<td>47</td>
</tr>
<tr>
<td><strong>Piezoelectric</strong></td>
<td>Au electrode/Acrylamide</td>
<td>L-Tryptophan</td>
<td>1–4 mM</td>
<td>8.8 μM</td>
<td>53</td>
</tr>
<tr>
<td><strong>Piezoelectric</strong></td>
<td>Au electrode/Methacrylic Acid</td>
<td>Quinine</td>
<td>10–1080 mg L$^{-1}$</td>
<td>2.04 mg L$^{-1}$</td>
<td>56</td>
</tr>
<tr>
<td><strong>Piezoelectric</strong></td>
<td>Au electrode/Methacrylic Acid</td>
<td>Saccharine</td>
<td>51–3420 mg L$^{-1}$</td>
<td>32.8 mg L$^{-1}$</td>
<td>56</td>
</tr>
</tbody>
</table>


**Table 2.** Modification type, measurement type, LOD, and detection range
6. Conclusion

As a result of these examples and studies, molecularly imprinted sensor systems have been developing, and they will continue to be developed. Just as we mentioned above, biological receptors are restricted to detect analyte by environmental parameters. Therefore, the combination of molecular imprinting technology and the chemical sensor technologies useful to be employed as bio-mimicking measurement system, and these combinations are easy to construct as well as they have a low cost causing them to become more prominent to focus on.

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