TÜRKİYE CUMHURİYETİ
ADANA ALPARSLAN TÜRKEŞ SCIENCE AND TECHNOLOGY UNIVERSITY

GRADUATE SCHOOL
DEPARTMENT OF BIOENGINEERING

DEVELOPMENT OF L-PROLINE IMPRINTED NANOFILM COATED SURFACE PLASMON RESONANCE SENSORS

SAYGIN ALTÜRK
M.Sc.

ADANA 2025

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SUPERVISOR
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ADANA 2025

DECLARATION OF CONFORMITY

In this thesis study, which was prepared following the thesis writing rules of Adana Alparslan Türkeş Science and Technology University Institute of Graduate School, I declare that I provide all the information, documents, evaluations and results in accordance with scientific ethics and moral codes without resorting to any means or assistance that would be contrary to scientific ethics and traditions. I also declare that I refer to all of the articles I used in this study with appropriate references and accept all moral and legal consequences if a situation is found contrary to my statement regarding my work.

12/06/2025

Saygın ALTÜRK

ÖZET

L-PROLİN BASKILANMIŞ NANOFİLM KAPLI YÜZEY PLAZMON REZONANS SENSÖRLERİNİN GELİŞTİRİLMESİ

Saygın ALTÜRK
Yüksek Lisans, Biyomühendislik Anabilim Dalı
Danışman: Prof. Dr. Gözde BAYDEMİR PEŞİNT
Haziran 2025, 69 sayfa

Prolin hastalıkların saptanmasında ve tanımlanmasında önemli bir biyobelirteç haline gelmiştir. Literatürde özofagus kanseri olan metabolik hastaların serum prolin seviyelerinin önemli ölçüde düşük olduğu belgelenmiştir. Ayrıca, kandaki prolin seviyesinin yükselmesiylede nadir görülen bir metabolik durum olan hiperprolinemi ortaya çıkar. Moleküler Baskılanmış Polimerler (MIP'ler), belirli bir hedef molekülü "kalıp" olarak kullanan ve yapılarında bu moleküle özgü tanıma bölgeleri oluşturan sentetik polimerlerdir. MIP'ler, yüzey plazmon rezonans (SPR) sensörlerinde tanıma bileşenleri olarak kullanılabilir. SPR biyosensörleri, ince bir metal film üzerinde veya yakınında kırılma indeksindeki değişiklikleri ölçerek hedef biyomoleküllerin hızlı, hassas ve ekonomik bir şekilde tespit edilmesini sağlar. Ayrıca, MIP'lerle birleştirildiğinde, sensörlerin seçiciliği ve hassasiyeti önemli ölçüde artırılabilir. Bu çalışmada, insan serumundan L-prolin molekülünü seçici olarak tespit etmek için altın SPR çipinin yüzeyinde L-prolin baskılı nanofilmlerin sentezlenmesi amaçlanmıştır. Nanofilm sentezinde kullanılacak en etkili ön-kompleks oranını belirlemek için Pro:VIM ile farklı molar oranlarda ön-kompleksler hazırlanmıştır. Poli (2-hidroksietil metakrilat) (PHEMA) bazlı Pro-MIP ve baskısız (NIP) SPR sensörleri sentezlenmiş ve taramalı elektron mikroskobu görüntüleri (SEM), Fourier Transform Kızılötesi Spektroskopisi (FTIR), Atomik Kuvvet Mikroskopisi (AFM) ve temas açısı ölçümleri ile karakterize edilmiştir. SEM analiz sonuçlarına göre polimerizasyonun yüzeyde homojen bir şekilde gerçekleştiği gözlemlenmiştir. Prolinin gerçek zamanlı tespiti için adsorpsiyon çalışmalarında çeşitli konsantrasyonlarda sulu L-prolin çözeltileri kullanılmış ve tespit limiti 0,5 µg/mL olarak elde edilmiştir. SPR izoterm parametreleri buna göre belirlenmiş ve sonuçlar Pro-MIP SPR sensörlerinin Langmuir izoterm

modeliyle daha uyumlu olduğunu göstermiştir. SPR sensörünün L-prolin için seçiciliği, yarışmacı moleküller L-histidin ve L-fenilalanin varlığında değerlendirilmiştir. Yapılan seçicilik çalışmaları sonucunda, prolin yarışamcı moleküller olan histidin ve fenilalanile göre sırayla 6,4 ve 7,5 kat fazla seçicilik gösterdi. Ayrıca, Pro-MIP SPR sensörlerinin adsorpsiyon kapasitesinde önemli bir düşüş olmadan 5 defaya kadar kullanılabileceği belirlenmiştir. Sonuçlar, L-prolini düşük tespit limitlerinde eş zamanlı olarak belirleyebilen bir biyosensör elde edildiğini gösterdi.

Anahtar Kelimeler: L-prolin, moleküler baskılanmış polimerler, yüzey plazmon rezonans sensörleri, nanofilm, kanser biyobelirteci.

ABSTRACT

DEVELOPMENT OF L-PROLINE IMPRINTED NANOFILM COATED SURFACE PLASMON RESONANCE SENSORS

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M.Sc., Department of Bioengineering

Supervisor: Prof. Dr. Gözde BAYDEMİR PEŞİNT
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Proline has become an important biomarker for the detection and identification of diseases. It has been documented in the literature that metabolic patients with esophageal cancer had significantly lower serum proline levels. In addition hyperprolinemia, a rare metabolic condition, occurs when the level of proline in the blood is elevated. Molecularly Imprinted Polymers (MIPs) are synthetic polymers that use a specific target molecule as a “template” and create recognition sites specific to this molecule in their structure. MIPs, can be used as recognition components in surface plasmon resonance (SPR) sensors. SPR biosensors provide rapid, sensitive, and economical detection of target biomolecules by measuring changes in the refractive index on or near a thin metal film. Furthermore, when combined with MIPs, the selectivity and sensitivity of the sensors can be significantly increased. In this study, aim was to synthesize L-proline imprinted nanofilms on the surface of gold SPR chip to selectively detect the L-proline molecule from human serum. Poly (2-hydroxyethyl methacrylate) (PHEMA)-based Pro-MIP and non-imprinted (NIP) SPR sensors were synthesized and characterized by scanning electron microscope images (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Atomic Force Microscopy (AFM) and contact angle measurements. According to SEM analysis results, it was observed that polymerization occurred homogeneously on the surface. Aqueous L-proline solutions at various concentrations were used in adsorption studies for the real-time detection of prolin and detection limit was obtained as 0,5 µg/mL. The SPR isotherm parameters were determined accordingly, and the results demonstrated that Pro-MIP SPR sensors were more compatible with the Langmuir isotherm model. Selectivity of the SPR sensor for L-prolin was evaluated in presence of competitor

molecules L-histidine and L-phenylalanine. In selectivity studies, proline showed 6.4 and 7.5 times higher selectivity than the competing molecules histidine and phenylalanine, respectively. In addition, it was determined that Pro-MIP SPR sensors can be used up to 5 times without significant decrease in adsorption capacity. Outcomes showed that a biosensor was obtained that could determine L-proline in low detection limits, simultaneously.

Keywords: L-proline, molecularly imprinted polymers, surface plasmon resonance sensors, nanofilm, cancer biomarker.

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NOMENCLATURE

ABBREVIATIONS

MIP : Molecularly Imprinted Polymer

MIT : Molecular Imprinting Technology

HEMA : 2-hydroxyethyl methacrylate

EGDMA : Ethylene glycol dimethacrylate

AIBN : Azobisisobutyronitrile

VIM : 1-Vinylimidazole

SPR : Surface Plasmon Resonance

SEM: Scanning electron microscope

FTIR : Fourier Transform Infrared Spectroscopy

AFM : Atomic Force Microscope

PBS : Phosphate - buffered saline

UV : Ultraviolet

ICONS

ΔR : Delta reflection

Rmax : Maximum SPR signal shift

C : Analyte concentration

ka : Binding rate constant

kd : Separation rate constant

KA : Binding constant

KD : Dissociation constant

1/n : Freundlich surface heterogeneity index

k : Selectivity coefficient

1. INTRODUCTION

Identification and quantification of amino acids as well as biochemical analysis are crucial methods, particularly for the diagnosis of metabolic diseases (Ohshima and Soda 1990). Proline is an amino acid and one of the 20 amino acids often found in animal proteins. Despite not having a fundamental amino group in their structure, proteinogenic amino acids are organic acids that are utilized in the production of proteins (Seebach, Beck, and Bierbaum 2004). When proline accumulates in the body and cannot be broken down by the enzymes proline oxidase or pyrroline-5-carboxylate dehydrogenase, a metabolic disorder known as hyperprolinemia develops. These enzymes produce proline in the proper way (Mitsubuchi et al. 2014). According to published research, the normal range for human free proline content in serum is 15.8–29.1 μg/mL. Hyperprolinemia causes an increase in proline levels in the blood and urine. In type I and type II hyperprolinemia, respectively, proline oxidase and proline-5-carboxylate dehydrogenase enzymes are not present. Type I hyperprolinemia causes kidney damage, and both types of the condition cause mental impairment (Namavar et al. 2021). Additionally, it has been shown in the literature that the proline level can be used to identify biomarkers for esophageal cancer (D'Aniello et al. 2020; Mustafa et al. 2011a). It has been discovered that patients have lower proline levels than healthy people (Zhang et al. 2012).

In this case, determining if L-proline is present in human body fluids is essential for both diagnosis and treatment. Due to the shortcomings of the existing approaches, particularly the simultaneous at low detection limits, a different strategy is needed that can accurately identify and analyze L-proline quickly and from a small number of samples. A variety of chromatographic methods are used to measure the amount of proline in biological fluids. These include gas-mass chromatography spectrometry (GC-MS), high performance liquid chromatography (HPLC) with fluorescence detection, ion exchange chromatography, and ninhydrin detection techniques (Delgado et al. 2005). Ion exchange chromatography and the methods for detecting ninhydrin are time-consuming and sample-intensive. HPLC and GC-MS need sample preparation in advance, and pre-derivatization of the column is a laborious and time-consuming procedure.To address these issues, the development of nanofim-coated SPR-based biosensors with L-Proline memory is recommended. Molecular imprinting is one technique for rapidly and precisely recognizing a target molecule in a complex environment. Molecular imprinting is defined as the process of creating ligand-selective recognition sites in

synthetic polymers by using a template (atom, ion, molecule, complex, or molecular, ionic, or macromolecular assembly, including microorganisms) to aid in the formation of recognition sites during the bulk phase assembly process by polymerization or polycondensation. Recognition must then take place in the spaces left by the templating species after some or all of the template is removed(Lofgreen and Ozin 2014a). A target molecule that interacts covalently or non-covalently with a functional monomer is used to produce molecular imprinted polymers (MIPs) (Chen et al. 2016). The target molecule and functional monomer pre-complex is dissolved in a solvent and kept in a complexation-friendly environment. The polymerization process is then initiated by the addition of the crosslinker and initiator. The removal of target molecules from the polymer matrix is a free process that creates binding sites and has exceptional selectivity against the target molecule, much to the immune system's key-lock mechanism. The polymer thus acquires molecular memory and identifies the target molecule that it can bind to. Drug carrier systems, biosensors, separation, and purification are just a few of the uses for MIPS that benefit from these features. The nanostructure of the materials significantly increases the MIP surface area when applied to a sensor configuration, thereby reducing sensitivity, increasing selectivity, and reducing analysis time (Saylan et al. 2017).

SPR-based biosensors are a straightforward and direct optical analysis method. Because of their high sensitivity, they can instantly detect biospecific interactions without the need for labeling (Kushwaha et al. 2018). It is made up of parts such as an optical unit, a liquid unit, and an integrated sensor chip. The liquid part of the SPR sensor is where biomolecular interactions take place, and the sensor chip serves as a physical barrier that separates the liquid cell from the optical unit (Nguyen et al. 2015). Real-time detection of non-covalent interactions between biomolecules, such as medicines and protein polysaccharides, as well as between proteins and viruses, enzymes and substrates, antibodies and antigens, proteins and nucleic acids, and proteins and DNA, is possible with SPR-based biosensors.

As part of the project's scope, L-proline adsorption from aqueous solutions conducted first, and the ideal binding conditions was determined. Later on, selectivity experiments was carried out with competing substances present. Lastly, the L-Proline imprinted SPR sensor's reusability will be examined. The detection of the L Proline molecule from human serum samples were examined as well.

The aim of this study is to analyze the L-Proline molecule, which is an important biomarker in esophageal cancer and hyperprolinemia, at low levels. L-Proline imprinted nanofilm-coated SPR based biosensors with high selectivity at detection limits development was aimed. In this way, specific recognition of L-Proline molecule directly from body fluids is intended.

2. LITERATURE REVIEW

2.1. Amino Acids

Proteins are made up primarily of amino acids. Both an amino group and a carboxylic group are present in amino acids. The function of the proteins that mediate messenger RNA (mRNA) translation is one of the many processes linked to gene expression that amino acids regulate. Protein is formed via the use of amino acids. Protein synthesis cannot take place in the absence of amino acids. Protein deficiency illness may develop as a result. A balanced diet that includes all of the essential amino acids is required. Certain amino acids are recognized to control insulin production from pancreatic β-cells both in vitro and in vivo, both acutely and chronically(Kimball and Jefferson 2006).

Amino acids are divided as acidic, basic and neutral amino acids. Some amino acids are not generated in the body and it is required to eat them in diet. Such types of amino acids are called essential amino acids. Some amino acids are produced by the body and are not required to be consumed through diet; these amino acids are referred to as non-essential amino acids. The body can synthesis certain amino acids, but it can only produce so many of them; they are known as semi-essential amino acids (Nomenclature and Di 2002). Amino acids aid in the synthesis of tissue proteins. Certain amino acids play a role in the synthesis of enzymes. Amino acids are the building blocks of hormones such as glucagon, growth hormone, and insulin. A single amino acid makes up thyroxin, nor-adrenaline, and adrenaline. Amino acids are also components of glutathione, a peptide that is physiologically active. Melanin is synthesized in part by amino acids. Research has shown that due to metabolic alterations, the amino acid balance in cancer patients frequently varies from that in healthy individuals (Maeda et al. 2010). Patients with liver cirrhosis may exhibit lower plasma levels of branched-chain amino acids, and dendritic cells (DCs) may not operate as well (Kakazu et al. 2007).

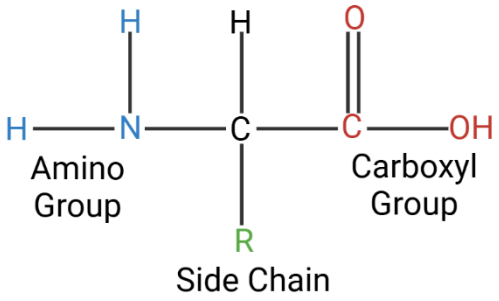

Figure 2.1. General Structure of Amino Acids (Created in https://BioRender.com).

2.1.1. Definitions of essential, non-essential, and functional Amino Acids

Traditionally, amino acids (AA) were categorized as nutritionally essential (indispensable) or non-essential (dispensable) for people and animals based on the requirements from the diet for nitrogen balance or growth. Essential amino acids (EAA) are those that the body cannot manufacture from scratch or that it cannot synthesize enough of in relation to needs. As a result, essential AA must come from the food in order to achieve optimal requirements.

The term "non-essential amino acids" (NEAA) refers to amino acids that the body is able to manufacture sufficiently in order to achieve optimal requirements. It should be understood that proper cell physiology and function depend on all 20 protein AA and their metabolites(Phang et al. 2008a). An AA's abnormal metabolism disrupts the body's equilibrium overall, hinders development and growth, and may even be fatal. A growing body of research reveals that some AA are significant regulators of critical metabolic pathways required for organism maintenance, growth, reproduction, and immunity in addition to their role as building blocks of proteins and polypeptides. This optimizes food utilization efficiency, increases protein accretion, decreases adiposity, and improves health (Wu et al. 2007) These amino acids; arginine, cysteine, glutamine, leucine, proline, and tryptophan are referred to as functional AA.

Table 2.1. Amino acids classification

Essential Amino Acids	**Non Essential Amino Acids**
Tryptophan	Glycine
Threonine	Tyrosine
Methionine	Proline
Isoleucine	Serine
Lysine	Alanine
Isoleucine	Asparagines
Histidine	Aspartic Acid
Phenylalanine	Glutamic Acid

2.2. Prolin

From a chemical and metabolic perspective, proline and its metabolite, hydroxyproline, are special amino acids. (Kaul, Sharma, and Mehta 2008). About 30% of all proteins in the body are collagen proteins, of which they comprise one-third of the amino acids. Proline is the amino acid, that is most needed per gram for the synthesis of all body proteins. Interest in proline metabolism and nutrition studies has grown (Phang, Liu, and Zabirnyk 2010). There is growing evidence that proline is a key regulator of a number of physiological and biochemical processes in cells. Proline, for instance, participates in redox reactions in both humans and animals as a signaling molecule, a sensor of the energy status of cells, and a generator of the free radicals superoxide anion and pyrroline 5-carboxylate (P5C)(Phang et al. 2008b, 2010). Additionally, proline is essential for the conceptus's growth and development as well as the differentiation of other cell types, such as embryonic stem cells. (Pistollato et al. 2010). With the important finding that proline is a major amino acid required for the synthesis of polyamines in the small intestine and placenta, which are crucial regulators of DNA and protein synthesis, as well as cell proliferation and differentiation, the crucial role that proline plays in fetal and neonatal nutrition was also reexamined.

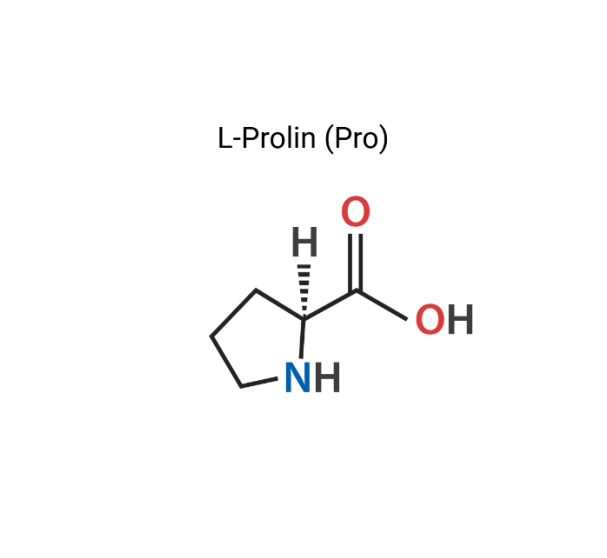

Figure 2.2. Structure of the proline (Created in BioRender. ALTÜRK, S. (2025) https://BioRender.com/e54z830).

Proline's α-imino acids are derived from its α-imino group. Proline's distinct ring structure sets it apart from other AA in terms of biological reactions, stiffness, and chemical stability. Post-translational hydroxylation occurs in proline because the hydroxylated residues appear after collagen polypeptides are synthesized. The process also occurs in other proteins that regulate cellular oxygen sensing and physiological responses to hypoxia (Chandel 2010).

Proline is involved in cell physiology and metabolism in a variety of ways. For instance, in the small intestine of newborn pigs and the placentae of gestating pigs and sheep, proline is a key nitrogenous substrate for the synthesis of polyamines. The fact that both organs exhibit high rates of cell proliferation and protein production makes this result noteworthy. Proline oxidase and ornithine decarboxylase can transform proline into polyamines. (Wu et al. 2005, 2008). Additionally, it is now known that proline and its metabolite P5C affect cellular signaling pathways and gene expression, both of which are critical for both health and illness. (Hu et al. 2008). Interestingly, proline may scavenge free radicals; this antioxidant property could help explain why proline concentrations increase significantly when cells experience oxidative stress. (Verbruggen and Hermans 2008). Moreover, new research indicates that proline might be involved in controlling the activation of the mammalian target of rapamycin (mTOR) pathway, which integrates signals from growth factors, cellular energy status, nutrients (such as glucose and AA), and different stressors to influence cell growth and function (Van Meijl,

Popeijus, and Mensink 2010). Thus, proline functions in concert with arginine, glutamine, and leucine to enhance protein synthesis in cells and tissues. (Wu et al. 2010).

Proline's roles in the physiology and metabolism of cells

- Control over cell division and gene expression.
- mTOR activation (combining growth factor and nutrition signals in cells)
- Proline communication through pyrroline-5-carboxylate, the free radical superoxide anion, and cellular
- Glutamate, polyamines, and protein synthesis all contribute to redox processes..
- Mammals produce arginine, which is especially crucial for newborns who are fed milk.

2.2.1. Proline Synthesis

In mammals, the synthesis of proline from arginine, ornithine, glutamine and glutamate varies depending on the cell, tissue, and species. Ornithine, aminotransferase, arginase, and P5C reductase are the enzymes that allow all mammals to generate proline from arginine. The organs that are quantitatively most active are the breast tissue, small intestine (in postweaning animals), liver, and kidneys (Wu et al. 2008). Proline, urea and ornithine are the main byproducts of arginine catabolism in breast tissue. There is no breakdown of arginine-derived proline in the lactating gland since proline oxidase activity is lacking from mammary tissue. This guarantees that the nursing mammary gland produces the maximum net amount of proline from arginine. Mammary tissue does not form proline from glutamine or glutamate since P5C synthase is likewise lacking from this tissue (O'Quinn, Knabe, and Wu 2002). Arginase thus contributes significantly to the production of proline in lactating breast tissue. Remarkably, in lactating breast tissue, P5C reductase activity is at least fifty times higher than P5C dehydrogenase activity, favoring the production of proline from arginine-derived P5C as opposed to glutamate and glutamine (O'Quinn et al. 2002).

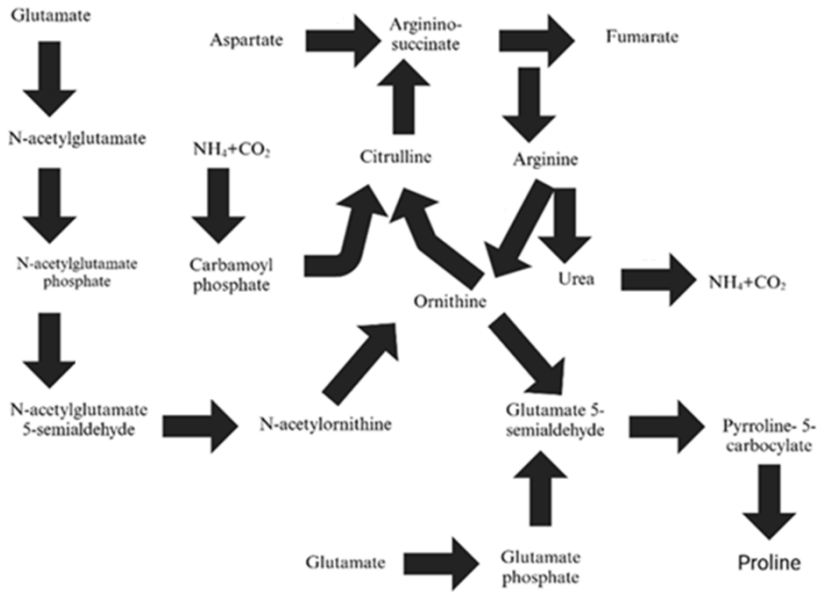

Figure 2.3. Characteristics of proline and its metabolites (Created in https://BioRender.com).

2.2.2. L-Prolin and D-Prolin

In both plant and animal proteins, proline residues undergo post-translational modification by the enzyme proline hydroxylase, resulting in the formation of 3- and 4-hydroxyproline. Certain bacteria use free L-proline to create hydroxyproline, which is then used to create secondary metabolites (Shibasaki et al. 1999). Of the four isomeric forms of 4-hydroxyproline, trans-4-hydroxy-L-proline (trans-4-L-Hyp) and cis-4-hydroxy-D-proline (cis-4-D-Hyp) seem to be the most prevalent. 4-hydroxyproline possesses two chiral carbon atoms (Gorres and Raines 2010). Numerous widely distributed glycoproteins and cell wall proteins in plants and algae are high in hydroxyproline. Alfalfa and several other plants contain additional proline derivatives, such as hydroxyproline betaine and proline betaine (N,N dimethylproline or stachydrine), which are typically connected to osmotic adaptation(Burnet et al. 2000).

Enantiomers are mirror-image isomers such as D- and L-proline. The structural and physiological distinctions between these enantiomers, which are commonly referred to as "chiral" forms, highlight the importance of stereochemistry in biochemistry and biology. The quality of molecules with mirror-image isomers that are not overlaid upon one another is known

as chirality. When it comes to amino acids, the primary cause of this chirality is the asymmetry of the alpha (α) carbon, which is the center carbon atom (Wang et al. 2015).

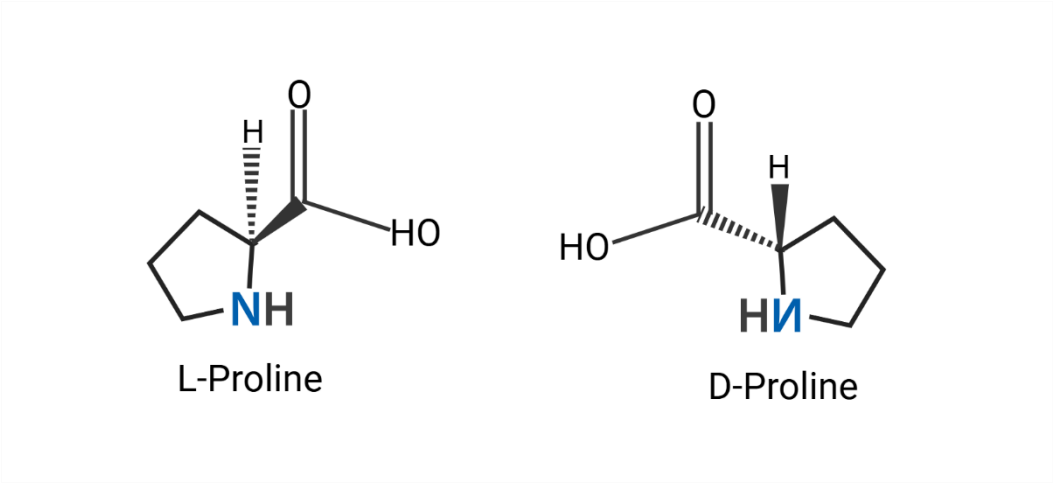

Figure 2.4. Molecular structures of L-Proline and D-Proline (Created in https://BioRender.com).

The two optical isomers of a single amino acid, known as L- and D-amino acids, have distinct optical activities but the identical physical properties (Schwabe 2004). The pair of isomers with optical properties can never overlap by rotation or other spatial operations because of one asymmetric C atom in their chemical structure. Generally speaking, amino acids are found in nature as L-amino acids, whereas their corresponding D-amino acids are synthetic, indigestible by living things, and possibly even hazardous to health(Lieu et al. 2020). However, some therapeutic medications and diets may require a considerable dose of D-amino acids. For example, D-amino acids were present in foods because dietary L-amino acids were racemized during procedures in the food industry; D-tryptophan was involved in the production of Tadalafil (Daugan et al. 2003). Because of this, it's critical to accurately and selectively identify the enantiomer of amino acids in a variety of fields involving amino acids, including food safety, medication production, and purification.

Additionally, measuring amino acids under diverse conditions is crucial for a number of reasons. For instance, proline is a multipurpose amino acid that is required for primary metabolism in both plant and mammalian tissues. In various organs, the functions and levels of toxicity of proline's enantiomers vary. For example, D-proline in the liver may produce severe

proximal tubular dystrophy and necrosis in the kidney, and periportal fibrosis and necrosis of liver cells. On the other hand, L-proline is an important neuronal modulator or transmitter candidate in the central nervous system(Lieu et al. 2020). Therefore, precise measurement of Proline enantiomers under various conditions is essential for examining its potential biological advantages or hazards.

2.2.2.1. L-Prolin In Human Health

L-proline is vital to human health since it supports a number of physiological functions that are necessary for our survival. Beyond its function in protein structure. Additionally, L-proline is a useful biomarker that sheds light on particular medical disorders and gives us a glimpse into our general health(Vettore, Westbrook, and Tennant 2021).

One of the twenty amino acids that are frequently found in animal proteins is proline, which is actually an amino acid. Mammalian proteins just contain the L type, or L-proline. It is not a necessary amino acid because the human body is able to generate it. In the cellular microenvironment, this amino acid is among the most prevalent, and it makes up around 25% of the residues in collagen, the primary protein in the extracellular matrix, along with hydroxiproline (Liu et al. 2012). The synthesis and maintenance of collagen would be hampered in the absence of L-proline, which could result in connective tissue disorders and skin-related problems (Mitran et al. 2015).

An essential component of wound healing is collagen. Its biosynthesis is intricate, requiring numerous sequential processes that are interconnected (Stein and Keiser 1971). In the wound area, fibroblasts are in charge of collagen synthesis. Fibroblast count in the wound tissue can be used as a predictor of wound healing (Savunen and Viljanto 1992). Collagen reinstallation and the deposition of freshly generated collagen fibers at the wound site determine the strength of the wound (Paul et al. 1997). Because it aids in the creation of collagen and other extracellular matrix components, L-proline is essential for wound healing. It facilitates tissue healing, ensuring that wounds heal properly and that the amount of scarring is reduced (Albaugh, Mukherjee, and Barbul 2017). L-proline is involved in many physiological processes, but it also acts as a biomarker, which is a quantifiable sign of a certain disease or physiological function.

The reference range for L-proline levels in blood in healthy adults is reported to be 15.8 to 29.1 micrograms per milliliter (μg/mL) (Liang et al. 2015). Proline metabolism has been linked to

cancer in several studies, and patients with renal cell carcinoma, oral cancer, colorectal cancer, and those at high risk of esophageal cancer (EAC) have all been found to have changed patterns of proline levels (Phang et al. 2012). Furthermore, metabolic individuals with esophageal cancer have been reported in the literature to exhibit considerably reduced serum proline levels. In this instance, L-proline detection is crucial for the identification, management, and monitoring of these illnesses. Consequently, more research is required to determine the precise function of this metabolite in cancer patients as well as the creation of appropriate tests for its detection using serum samples.

Blood levels of the amino acid proline are increased in hyperprolinemia, a rare metabolic condition (Namavar et al. 2021). Hyperprolinemia comes in two primary forms: type I (HPI) and type II (HPII). An autosomal recessive inborn defect of the proline metabolic pathway causes each kind.

Type I Hyperprolinemia (HP-I): The enzyme pyrroline-5-carboxylate dehydrogenase (P5CDH), which is involved in the conversion of pyrroline-5-carboxylate (P5C) to L-glutamate, is deficient in this variant of hyperprolinemia. Proline levels rise as a result of P5C building up and being converted to proline. Most of the time, type I hyperprolinemia is thought to be a benign illness with little or no symptoms (Namavar et al. 2021).

Type II Hyperprolinemia (HP-II): Hyperprolinemia type II is another name for hyperprolinemia (HPII). A lack of Δ-1-pyrroline-5-carboxylate (P5C) dehydrogenase activity is the source of HPII (Anon n.d.-d; Mitsubuchi et al. 2008). Neurological symptoms, intellectual incapacity, developmental delays, and behavioral problems might result from this type of hyperprolinemia (Namavar et al. 2021)

It's crucial to remember that hyperprolinemia is an uncommon disorder, and that afflicted people's symptom intensity can vary greatly. For patients with Type II hyperprolinemia, early identification and effective treatment are essential to maximizing long-term results and quality of life (Namavar et al. 2021).

2.2.2.2. Quantitative analysis of L-Proline

Multiple metabolomics studies have demonstrated the function of amino acids in various disorders, including cancer, by altering the blood concentrations of these amino acids (Scioscia, Snyderman, and Wagner 1998). Furthermore, recent research has indicated the significance of proline metabolism and its connection to cancer (Phang et al. 2012) and some patients with renal cell carcinoma have been observed to have changed proline level patterns (Catchpole et al. 2011; Mustafa et al. 2011b).

Several previously described chromatographic techniques that can identify proline in biologic fluids were found through a literature search. The conventional techniques, which involved ninhydrin detection and ion-exchange chromatographic separation, took a lot of time (Anon n.d.-a, Anon n.d.-b). Gas chromatography–mass spectrometry or high-performance liquid chromatography (HPLC) with fluorescence detection were the two techniques used to create further approaches. Pre-column derivatization was necessary for all of those procedures, which meant labor and time intensive sample preparation. Thus, a quick, easy, precise, and trustworthy technique for measuring the amount of L-proline in human serum needs to be developed.

2.3. Surface Plasmon Resonance (SPR)

Over the past 20 years, surface plasmon resonance (SPR) sensing technology has been widely used for biological and chemical analyte detection, environmental monitoring, and medical diagnostics since the SPR was initially applied in 1982 for gas detection and biologic sensors (Shankaran, Gobi, and Miura 2007). Surface plasmons, also known as propagating surface plasmons (PSPs) and localized surface plasmons (LSPs), are coherent oscillations of free electrons at the interfaces between metal and dielectric (Maier 2007). PSPs can be excited on metallic surfaces using a variety of techniques, including diffraction gratings, optical fiber couplers, Kretschman and Otto prism couplers, and optical waveguide couplers (Suryanarayana et al. 2022) Alternatively, LSPs can be excited on metallic nanoparticles, which can both cause a strong enhancement of the electromagnetic field in the near-field region (resonance amplification). This has a wide range of applications, including biomolecular interaction detection, surface-enhanced Raman scattering (SERS), fluorescence enhancement, refractive index (RI) measurement, and so forth (Kihm 2010).

Since their initial application, SPR biosensors have advanced quickly as a useful substitute for biological interaction analysis.

2.3.1. Principle of SPR

The majority of SPR sensing devices are created using a similar methodology, which includes optical sensors, microfluidic chips, sampling systems, and software for data collection and processing (Anon n.d.-c; Kooyman et al. 2008). Usually, continuous flow for kinetic studies and stopped flow for affinity testing allow sample delivery at the sensor surface to circumvent mass transport limitations. (Anon n.d.-c; Steiner 2004). Only material that is close to the chip surface (up to 300 nm) can trigger the SPR sensor (Olaru et al. 2015). Therefore, attaining precise, repeatable sample delivery and a markedly decreased sample volume depends heavily on the microflow cell's design (Wang et al. 2013). A tightly controlled isothermal regime should also be maintained because temperature changes have a major effect on the kinetics and affinity properties of biomolecular interactions and also the sensitivity of the SPR sensor response. (Scarano et al. 2010).

The essential components of an SPR sensor are an imaging optical system, a photodetector, optical coupling elements, and a light source and associated optical system. (Mariani and Minunni 2014). To improve SPR and SPR methods sensitivities and resolution a number of formats have recently been created, starting with the arrangement of the coupling components and the kind of light wave modulation.

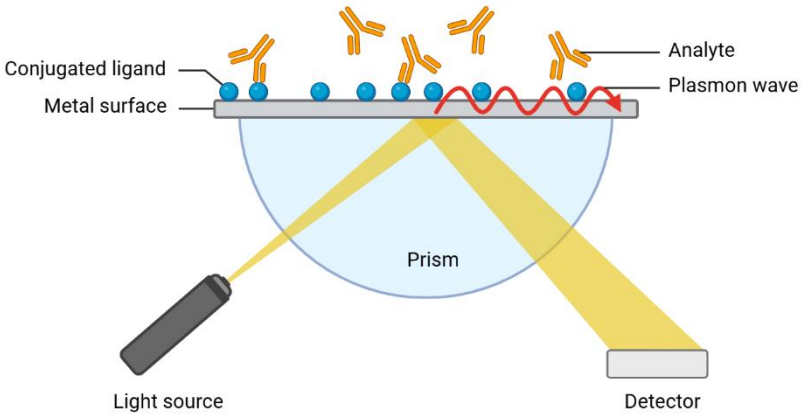

Figure 2.5. The principle of SPR (Created in https://BioRender.com).

2.3.2. SPR Biosensors

A biosensor is a device that combines biological recognition elements such as proteins, nucleic acids, antibodies, or enzymes as capture agents. These elements interact with the target analyte and, via a transduction element, produce a quantifiable signal. Recently, the surface plasmon resonance (SPR) technology has been widely utilized to identify pathogens such as bacteria, viruses, nucleic acids, toxins, allergens, teliosporic antigen, and antibodies, as well as to monitor the interaction between two binding molecules (Souto et al. 2015). The SPR biosensor offers a unique possibility for the quick and affordable detection and identification of target biomolecules by measuring the change in refractive index at or near a thin metal film surface (Situ et al. 2010). It also enables the direct, real-time, label-free, and quantitative detection of interactions (Zhang et al. 2022). Clinical and medical diagnostics has made use of SPR sensors, and research is now being done to create assays that quantify antigen-antibody interactions. SPR, which measures even low concentrations of the target analyte in a test sample, is emerging as one of the fastest evolving methods to assist in the identification of biomarkers. Traditional techniques for detecting macromolecules include PCR, culture, and enzyme immunoassays; however, these assays take a lot of time. These techniques have recently been superseded by

SPR technology (Prabowo et al. 2017). Sensitivity tests, however, are necessary for bacteria, viruses with extremely low titers, and low molecular weight targets. Modified gold nanoparticles and nanostructured substrates can improve the sensitivity and the detection limit of SPR sensors (Puiu and Bala 2016).

Figure 2.6. SPR-based biosensor.

2.3.2.1. Application Areas of SPR Biosensors

Surface Plasmon resonance, or SPR, has been shown to be useful as an optical biosensor by Liedeberg and Nylander in 1982 (Semerdjian-Rouquier, Bossi, and Scatton 1981). Since then, surface chemistry has benefited from SPR, creating a shared platform for chemistry, physics, and biology (Alves, Park, and Hruby 2005). Several thousand academic, industrial, and technological research SPR has been used as an optical biosensor in literature. SPR has been widely used as an optical biosensor and has been marketed by many firms under various brand names (Lee et al. 2012). Utilizing SPR biosensors has become more common in health science investigations, basic biology research, clinical diagnostics, environmental monitoring, drug discovery and agricultural farming. In clinical laboratories, the use of SPR is rapidly increasing in the field of quantitative analyses for point-of-care (POC) diagnostics, including therapeutic drug monitoring (TDM) for dosage control, mutation detection, immunoassay analysis and toxicity risk management to improve drug therapies with exceptional exceptional repeatability and reusing performance.

Real-time, label-free investigations of the kinetics and affinity of bimolecular binding are possible with surface plasmon resonance (SPR). SPR is clearly superior to radioactive or fluorescent marking techniques since it is less expensive, allows for direct determination of binding constant and affinity, and uses less reagents. Additionally, labels may make binding more challenging (Im et al. 2009).

Following the completion of the Human Genome Project (HGP) and the development of quick and inexpensive genomic sequencing, the focus has moved from studying genes at the genome level to analyzing proteins at the proteome level. This involves networks of interactions with various proteins that take place within cells. Investigation of these interaction networks identifies roles for the as-yet-uncharacterized proteins (Im et al. 2012). Modified SPR devices with a large field of view, multiplexing capability, high resolution images, and an easy-to-use optical design are needed to accomplish these goals. Proteomics' rise to prominence and the demand for high-throughput analysis have prompted the creation of SPR platforms with microarray analytic capabilities.

Because of their strong and adaptable capacity to enhance and control optical signals, the plasmonic properties of metallic nanoparticles (NPs), such as Ag NPs and Au, as well as the plasmonic relationship that exists between them, are extremely attractive. The robust optical

properties of these plasmonic devices promise a variety of biosensing applications. While a flat gold film in a conventional prism-based SPR is lighted at a high angle in complete internal reflection mode, gold-film nanoholes can start SPR directly with regular incident light and a straightforward illumination setup from a typical microscope. (Menezes et al. 2010). In addition to producing SP waves, nanoholes can function as tethered lipid vesicles, scaffolds for pore-spanning lipid membranes, nanowells to enclose supported lipid bilayer membranes, native cellular membranes, nanofluidic-based biomolecule transport channels, and electrodes for electrochemical sensing (Im et al. 2010).

At the intersection of micro-nanoscale engineering, microfluidics offers several benefits for POC techniques of infectious illness monitoring and detection, including adaptability, quick outcomes, and low fabrication costs. Furthermore, combining micofluidics platforms with optical imaging systems combines the benefits of lab-chip platforms with optical technology. (Tokel et al. 2015). Rolling circle amplification (RCA), nanostructured substrates, and tags modified with nanogold can all be used to increase the sensitivity and detection limit of traditional SPR devices. This could be applied to the analysis of food-related industries, environmental pollutants, and biomolecules (Lindquist et al. 2012).

2.3.2.1.1. SPR imaging

SPR imaging uses a video CCD image to visualize the entire biochip without labeling. Because of this construction, the biochips may be produced in an array fashion, with every active location (spot) providing SPR information simultaneously. Conventional SPR imaging research collects data using fixed angle, angle scanning, or wavelength scanning. (Linman et al. 2011). An extensive beam of homogeneous polarized light generated by a laser diode operating at a certain wavelength covers the whole functionalized surface of an SPRi-chip positioned within the instrument detecting chamber. Up to a few hundred active spots can be captured in real-time by a high-resolution CCD video camera in an array format (Nguyen et al. 2015).

Moreover, Corn et al. used the method for DNA hybridization detection and oligonucleotide arrays (Malic et al. 2007). Nelson et al. later used near infrared (NIR) excitation wavelength to enhance intensity SPR imaging performance. SPR pictures with high contrast have been displayed in the NIR wavelength range (800–1,152 nm) (Brockman, Nelson, and Corn 2000). The laser fringes seen in traditional SPR imaging setups are also eliminated by using an

incoherent white source and a narrow band-pass filter.(Li, Lee, and Corn 2006) The NIR SPR imaging setup has also been shown to be effective for protein biomarker screening and the detection of epitope–antibody, protein–carbohydrate, protein–protein, protein–DNA, and protein–aptamer interactions .

2.3.2.1.2. Combined SPR and electrochemical impedance spectroscopy (SP–EIS)

Combining SPR with other analytical methods has been suggested recently as an effective means of obtaining complementing parameters for sample analysis as well as raising sensitivity and resolution (Kuai et al. 2019). For example, electrochemical impedance spectroscopy, or EIS, label-free SPR sensing, also known as linking, multiplexing readouts, has several advantages over conventional detection methods and is an even stronger instrument than either optical or electrochemical sensing alone (Abbas, Linman, and Cheng 2011). Electrical biosensors based on EIS, like SPR, may operate label-free and don't require any specific reagents. There have been several proposed EIS biological sensing systems, and some of them use interdigitated electrode (IDE) arrays made with traditional microfabrication techniques (Anon n.d.-e; Shul'ga et al. 1994). The complimentary application of these two methods, SPR and EIS, can aid in the development of novel sensing platforms and biochemical procedures for the rapid, simple, and label-free identification of biomolecules with enhanced performance and sensitivities. (Choi and Hillier 2010). For example, dielectrophoresis (DEP) using biochips or electrokinetic label-free screenings can improve chip-based immunoassay methods for microbial findings in healthcare diagnostic equipment (Krishnamoorthy et al. 2010).

2.3.2.1.3. Multi-parameter SPR

Otto created the first SPR sensor structure in 1968 by combining a prism with a metal film. Due to its great sensitivity, this sensor structure garnered a lot of attention (Otto 1968). Nevertheless, the Otto structure's design makes the sensor's overall system more complex. Consequently, Kretschmann greatly decreased the structural complexity and enhanced the Otto structure in 1971 (Kretschmann 1971). Nevertheless, the prism-based SPR sensor's huge bulk and requirement for intricate optical and mechanical components make it unsuitable for remote

sensing (Rakibul Islam et al. 2020). Hassani et al. created the first surface plasmon resonance sensor based on photonic crystal fiber in 2006 by combining SPR sensing with photonic crystal fiber (PCF) (Hassani et al. 2006) This sensor features a high sensitivity, outstanding single-mode properties, easy phase matching, and a versatile form. Nevertheless, this arrangement places the metal film and the liquid channel in the photonic crystal fiber's cladding air hole, which is challenging to create and demands a high procedure. In order to significantly reduce the practical operation complexity and process challenges, Ming Tian et al. proposed a separate cladding coat model based on D-type PCF in 2012. This model placed the metal and liquid layer outside of the cladding (Tian et al. 2012).

However, the aforementioned sensors are only able to identify a single parameter. By enabling the detection of several parameters in a single sensor, the sensor's integration may be effectively enhanced. A temperature and refractive index sensor based on D-shaped photonic crystal fiber was proposed by Shiwei Hua et al. in 2015 (Shi, You, and Wu 2015). Temp-sensitive liquid toluene is poured into an air gap in the cladding, and the direction coupling effect is used to measure the temperatures. Hai Liu et al. (2017) proposed a two-parameter optic sensors that utilized directed coupling resonance for PCF temperature and magnetic field. (Liu et al. 2017). In 2019, Ying Yu et al. proposed a two-parameter sensors using a D-type PCF and a magnet field and sensor for temperature (Ying et al. 2019). After being placed on the side polishing plane, the magnetic fluid is filled with ethanol in the air hole. Under 30–270 Oe and 5–65 °C circumstances, the sensor can show a magnetic field response of 0.21 nm/Oe and a temperature sensitive of −1.25 nm/°C.

2.3.2.1.4. SPR–MS

In complicated biological matrices, mass spectrometry (MS) can identify and detect proteins and their smaller peptide fragments, but only in conjunction with multidimensional separation techniques. The main use of these SPR biosensors is the characterization of protein interactions between solution-borne analytes (i.e., proteins) and surface-immobilized ligands (e.g., antibodies). Proteins that have been affinity-retrieved on the SPR sensing surface can be further examined by mass spectrometry (MS) either immediately from the sensor/chip surface or separately after an elution and microrecovery process, due to the nondestructive nature of SPR detection. Proteins that are otherwise undetectable by SPR sensing alone can be revealed by the

ensuing MS analysis. These proteins may be complexed (with other molecules) or have variations due to post-translational modifications. SPR-MS, also known as BIA/MS, or Biomolecular Interaction Analysis/MS, is the term used to describe the combination of SPR biosensors and MS (Xue, Bai, and Liu 2019). Biacore flow cell-based biosensors, which use up to four distinct ligand-immobilization sites with an area of 1 mm^2, have been used for the majority of SPR-MS investigations. But rather than being the result of technological constraints, these size and site-count restrictions are a result of the design of the instruments themselves. It is generally acknowledged that high-density functional protein arrays are a good high throughput, miniature substitute for traditional immunoassays (Ishige, Honda, and Shimizu 2005).

2.4. Molecular Imprinting

"The construction of ligand-selective recognition sites in synthetic polymers where a template (complex, ion, molecule, or a molecular, ionic, atoms, or macromolecular assembly, including micro-organisms) is utilized for facilitating recognition site formation following the covalent assembly of the bulk phase by a polymerization or polycondensation process, with after removal of some or all of the template being necessary for recognition to take place in the spaces vacated from the templating species" is how molecular imprinting is defined. (Whitcombe, Kirsch, and Nicholls 2014). The design, production, characterization, and use of molecularly imprinted polymers (MIPs) have advanced steadily in recent years, which is indicative of MIT's gradual maturity and the scientific community's strong interest in it. In contrast to other recognition systems, molecularly imprinted polymers (MIPs) have attracted a lot of interest and become more attractive in a number of domains, such as chemo/biosensing, drug delivery,artificial antibodies, and catalysis, purification and separation, and degradation due to their high physical stability, ease of preparation, remarkable robustness, and affordability. Structure predictability, recognition specificity, and application universality are the three main distinctive characteristics of MIPs (Chen, Xu, and Li 2011).

2.4.1. Fundamentals of MIPs

Molecular imprinting involves polymerizing a cross-linker and a functional monomer around a molecular template (Yan and Kyung 2006a). First, a specific template molecule and a complementary functional monomer produce template–monomer complexes that are precisely arranged to distinguish different molecular imprinting techniques.
(Lofgreen and Ozin 2014b). Around the complex, a crosslinking polymerization procedure is then carried out. When the template molecule is recovered, It's crucial to note that the imprinted sites feature a three-dimensional arrangement of porosity that match the templates' functional group geometries and positions.

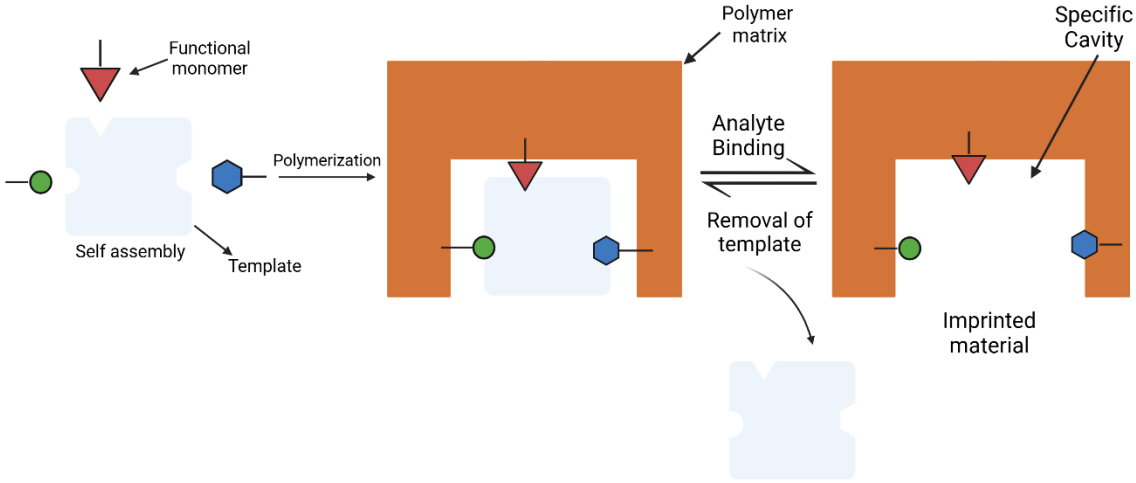

Figure 2.7. Molecular imprinting process (Created in https://BioRender.com).

Since covalent imprinting is stoichiometric, It ensures that only the imprinted cavities contain functional monomer residues. The other two primary techniques for creating MIPs are based on noncovalent interactions between the template and the functional monomer. It is a common procedure that frequently makes use of Schiff's base, ketals/acetals, and boronate esters in easily reversible condensation processes (Woźnica, Sobiech, and Luliński 2023). Because there are fewer reversible condensation processes, covalent imprinting is thought to be a less versatile technique. Furthermore, because of the strong covalent bonds that cause sluggish binding and dissociation, it is particularly difficult to attain thermodynamic equilibrium (Alexander et al. 2006).

Van der Waals forces, hydrogen bonds, ionic contacts, and π-π interactions can all be used to promote noncovalent imprinting. In nonpolar solvents, hydrogen bonding which frequently happens between primary amines and methacrylic acid (MAA) groups is the most prevalent interaction (Derazshamshir 2021). Since noncovalent imprinting is so easy to use and binds and removes quickly, it has emerged as the most often used generic synthesis approach. Noncovalent imprinting, on the other hand, is not very robust since it is vulnerable to even minute disruptions of the interactions holding the complex together (such as the presence of water) (Lofgreen and Ozin 2014b). A new technique known as semicovalent imprinting has emerged to combine the speedy target absorption of noncovalent imprinting with the stability of covalent imprinting. This approach provides a middle ground where the functional monomer and template are coupled covalently, but template rebinding is dependent on noncovalent interactions (Chen et al. 2011).

2.4.2. Essential elements of molecular imprinting

A template, a functional monomer, a cross-linker, a polymerization initiator, and a solvent (porogen) are the usual components of a MIP synthesis technique. Many attempts have been made to manufacture MIPs with improved qualities because the temperature and duration of the polymerization reaction, as well as the kind and amount of solvent, initiator, monomer, and cross-linker, all affect the polymerization reaction.

2.4.2.1. Target templates

Generating MIPs with affinity and specificity similar to biological receptors is the ultimate goal of molecular imprinting, with the hope of someday replacing these biological entities in practical applications. Generally speaking, An ideal template Three requirements should be met by a molecule: it has to possess functional groups that do not obstruct polymerization, exhibit exceptional chemical stability during the polymerization reaction, and have functional groups that enable it to form complexes with functional monomers. (Derazshamshir 2021). MIPs have been effectively used thus far to recognize and detect a variety of tiny chemical compounds. However, because the metal ions have identical charges, ionic radii, and other characteristics, low selectivity is frequently achieved when the metal ion itself serves as an imprinting template (Zhang et al. 2014). Furthermore, reports have been made about big structured species for MIPs,

including viruses, proteins, and cells (Green and Sambrook 2019). Protein imprinting and other biomacromolecule imprinting is still quite difficult, though (Liu and Ulbricht 2017).
The most recent comprehensive review paper on IIPs, which discusses anion imprinting, ion-mediated secondary imprinting, rare earths, metalloids, actinides, transition elements, and main group elements, is recommended reading. (Fu et al. 2015).

Table 2.2. Common molecular imprinting target templates

Type	**Examples**
Small Organic Molecules	These consist of pharmaceuticals, environmental contaminants, and pesticides and herbicides. Ibuprofen, atropine, and atrazine are a few examples.
Proteins and Peptides	Therapeutic proteins and disease markers are two examples of the biological uses for these. Insulin, hemoglobin, and myoglobin are a few examples.
Nucleic Acids	Sequences of DNA and RNA are utilized in genetic analysis and diagnosis.
Vitamins	Additionally, vital vitamins like vitamin D and B12 are utilized as templates.
Metabolites	Common targets include metabolites from a variety of biological activities, including cholesterol and glucose.

2.4.2.2. Functional monomers

By supplying functional groups, the functional monomer helps the template create a pre-polymerization complex. Therefore, before polymerization, it's crucial to choose a functional monomer that can interact with the template in a robust way and generate certain donor-receptor or antibody-antigen complexes. The selectivity and potential uses of MIPs are somewhat constrained by the limited number of functional monomers available for molecular imprinting. The creation of novel functional monomers that can establish robust interactions with templates is of utmost importance. A functional monomer often consists of two different kinds of units. A vinyl double bond and a silicon hydroxyl are examples of polymerizable units, whereas recognition units are additional types of units. As a result, several complex ligands that have been altered by silicon hydroxyl and vinyl group have been created (Sun et al. 2024).

Diacetylene monomers have been utilized more recently to manufacture MIPs because photopolymerization without the need for an outside catalyst or initiator makes the imprinting process more feasible.

In order to bind two receptor sites concurrently, König et al. inserted photopolymerizable diacetylene tagged dinuclear zinc cyclen receptors (Zn2PCDA, compound 36) in the fluid membrane. These receptors were pre-organized in the presence of a peptide template. Organized arrays of receptor sites formed patterns on the vesicle surface as a result of light-induced polymerization of polydiacetylene. This imprinting approach outperforms the current approaches, which mostly rely on the use of hybrid biosensors, metal complex fluorophore conjugates, or combinatorial, pattern recognition-based methods (Banerjee and König 2013).

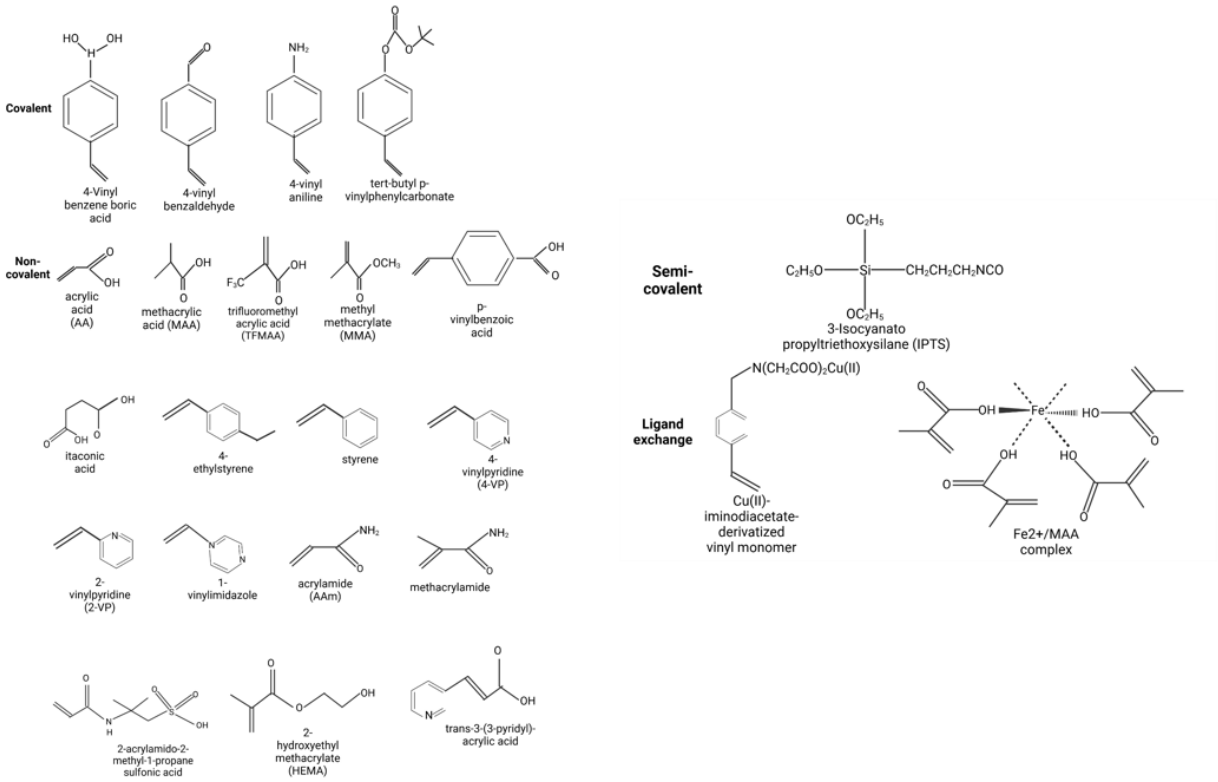

Figure 2.8. Common functional monomers utilized in processes involving molecular imprinting (Created in https://BioRender.com).

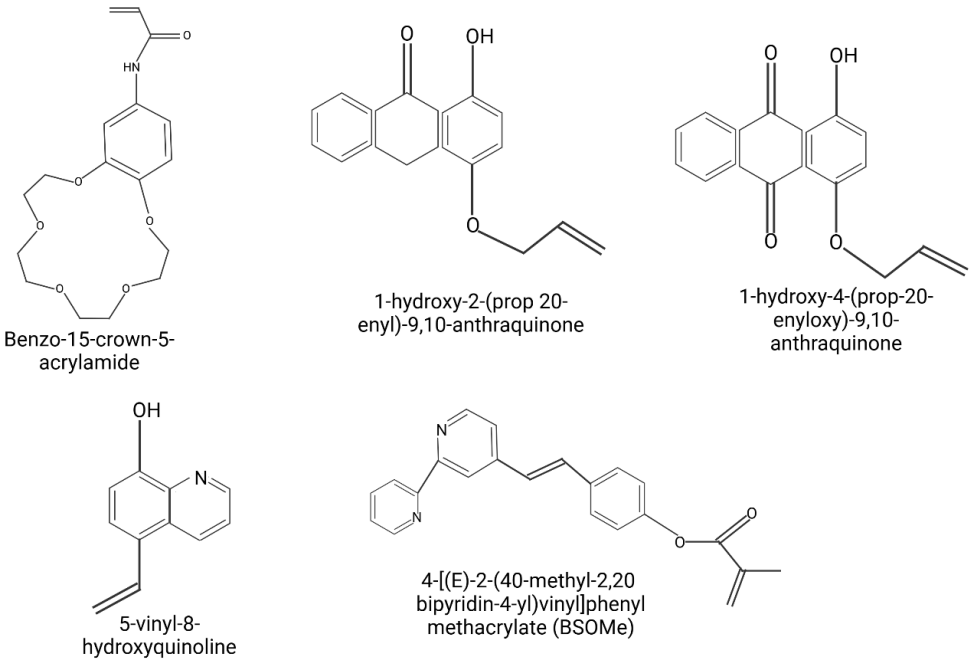

Figure 2.9. New useful monomer chemical structures for molecular imprinting (Created in https://BioRender.com).

2.4.2.3. Cross-linkers

Functional monomers are fixed around template molecules by a cross-linker during the polymerization process, resulting in the formation of a strongly cross-linked stiff polymer even after the templates are removed. The kind and concentration of the cross-linker have a significant impact on MIPs' selectivity and binding ability (Yan and Kyung 2006b). In general, too high of a cross-linker concentration will result in fewer recognition sites per unit mass of MIPs, whereas too low of a cross-linker concentration will result in unstable mechanical characteristics due to the low degree of cross-linking. However, the limited number of cross-linkers that are currently on the market and the tight constraints that their molecular architectures must meet limit their uses. New cross-linkers are desperately needed to synthesis MIPs with robust diffusion-controlled behavior.

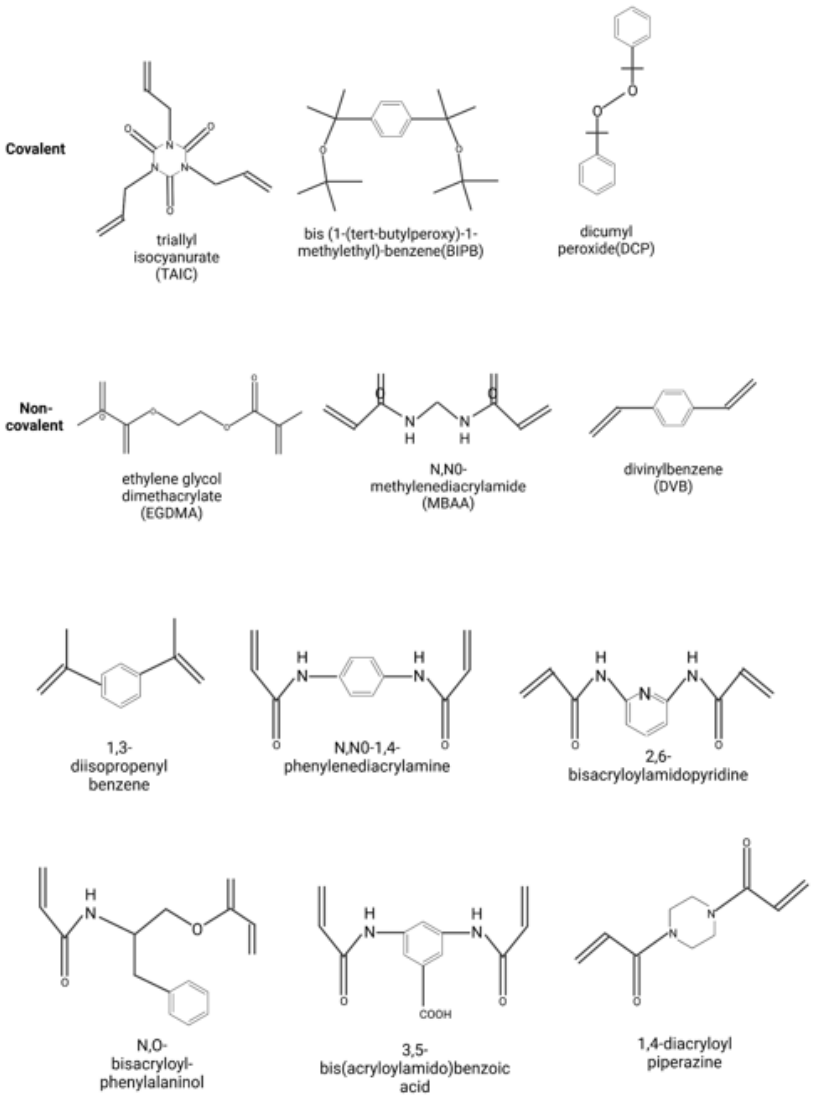

Figure 2.10. Common cross-linkers used in free radical polymerization and their chemical structures. (Created in https://BioRender.com).

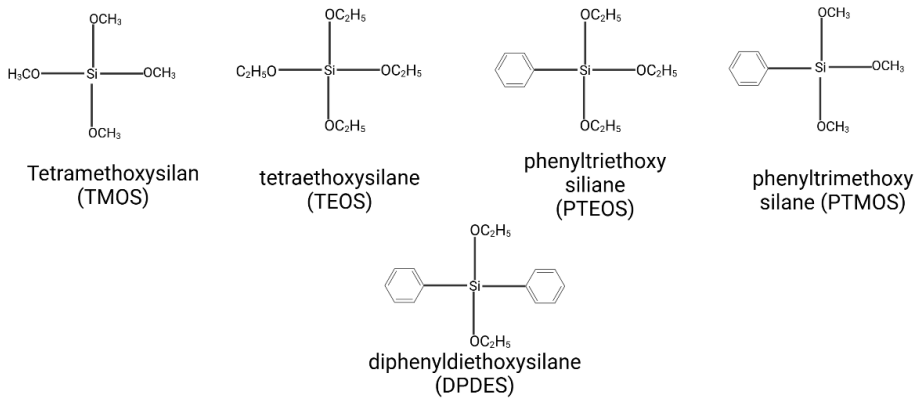

Figure 2.11. Common cross-linkers' chemical structures for sol–gel process (Created in https://BioRender.com).

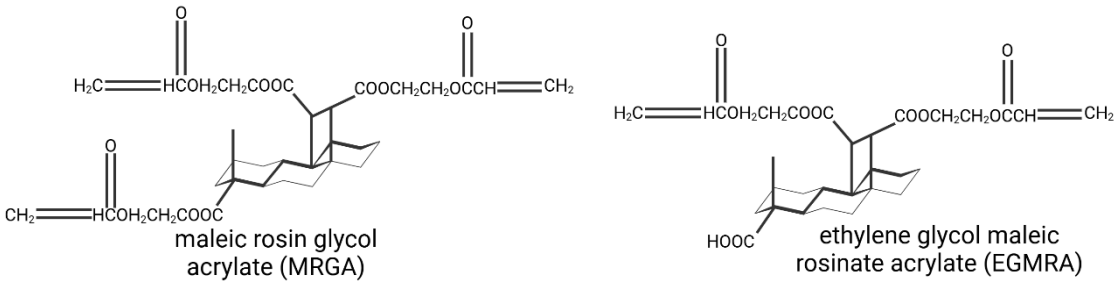

Figure 2.12. Chemical compositions of new cross-linkers (Created in https://BioRender.com).

2.4.2.4. Porogens

Porogens, sometimes referred to as porogenic solvents, are frequently used as dispersion media and pore-forming agents during the polymerization process. Thus, they are also crucial to polymerization. Typically, 2-methoxyethanol, dichloroethane, tetrahydrofuran (THF), acetonitrile, chloroform, N,N-dimethylformamide (DMF), methanol, and toluene are the solvents utilized in MIP production (Gladis and Rao 2004). The polarity of porogens may affect the chemical relationship with the functional monomer and the template molecule and, in turn, the adsorption properties of MIPs, especially in instances involving non-covalent interactions. To achieve good imprinting efficiency in non-covalent imprinting, non-polar and less polar organic solvents including toluene, acetonitrile, and chloroform are frequently utilized. This is because the types of solvents used have an impact on the morphology and adsorption properties of polymers. The use of theoretical simulations is crucial to assess the solvent and monomer selection procedures for molecular imprinting and to get insight into MIP selectivity. Four solvents were used in Saloni et al.'s study of the impact of solvents on monomer–template binding energy: methanol, acetonitrile, chloroform, and acetone. Additionally, all solvent, vibrational frequency, and structural calculations had been performed using density functional theory (DFT) (Saloni et al. 2021).

2.4.2.5. Initiators

As far as we are aware, photopolymerization, electropolymerization, and free radical polymerization (FRP) are the common methods used to create the great majority of MIPs. FRP can be started for a variety of functional groups and template structures by either thermal or photochemical means. Among them, the decomposition temperatures of 50–70 °C are the most convenient for using azobisisobutyronitrile (AIBN). It is crucial to remove the dissolved oxygen from polymerization solutions as soon as possible before proliferation occurs in order to guarantee the polymerization reaction. Additionally, oxygen can be removed by bubbling an inert gas such as argon or nitrogen.

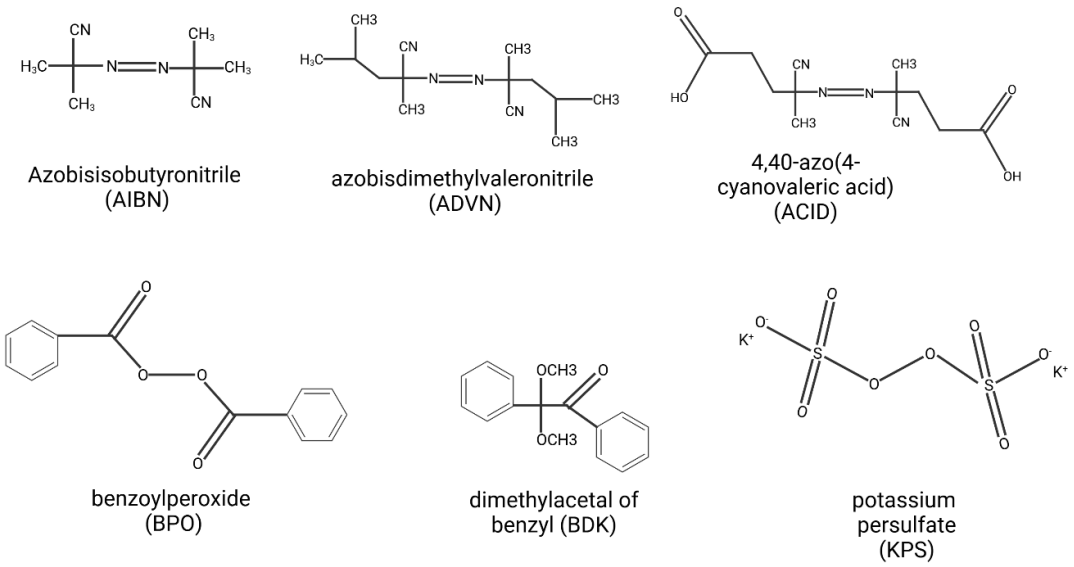

Figure 2.13. Chemical compositions of typical molecular imprinting initiators (Created in https://BioRender.com).

2.4.3. Preparation procedures

Making the right preparation technique choice is essential to producing MIPs with the desired qualities. Generally speaking, sol-gel and free-radical polymerization are the mechanisms of MIP preparation. The former is more widely used and accepted. The most popular type of free radical polymerization, bulk polymerization, lowers the MIP binding capacity relative to theoretical values by using mechanical grinding and filtering to produce tiny particles. Moreover, a lot of template molecules are required for this approach. More complicated and advanced polymerization processes have been developed to address these limitations of bulk polymerization to produce a variety of MIPs, including membranes, in situ manufactured monoliths, MIP particles, and molecularly imprinted monolayers. Suspension polymerization is a very easy process for making porous MIPs. Mineral oil, perfluorocarbon liquid, and the traditional water medium can all be utilized as the continuous phase for suspension polymerization. However, because the dispersing medium is disturbed, the method produces a

wide range of sizes from micrometers to millimeters and exhibits poor recognition, making it unsuitable for applications involving solid phase extraction (SPE) (Shah et al. 2022). Emulsion polymerization, which suffers from the disruption of surfactant residues, is a useful technique for producing high yield, monodispersed MIP particles, either in water-in-oil (W/O) or oil-in-water (O/W) (Badawy et al. 2022).

Multi-step swelling, also known as seed polymerization, has been used to directly create and modify in situ controlled diameter spherical MIPs. Despite the lengthy multistep process, the possibility of imprinting disruption and the decreased selectivity from the used aqueous suspensions, the particles generated by this approach are very monodisperse in both shape and size and are perfect for chromatographic uses (Chen et al. 2022).

Precipitation polymerization requires more organic solvents and more stringent reaction control than bulk polymerization because of the significant effects of stirring speed, temperature, and solvent polarity on the size of the polymer particles.(Kang et al. 2021). Additionally, monolithic MIPs have been created inside a chromatographic column using a straightforward, one-step in situ free-radical polymerization procedure that eliminates the need for grinding, sieving, and column packing (Faraji, Yamini, and Rezaee 2010; Yang et al. 2012). Tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS), which are precursors of tetraalkoxysilanes, hydrolyze to form a colloidal solution (a sol), which then polycondense to form silica materials (or gels) with abundant cross-linking (Lofgreen and Ozin 2014a). The sol gel process can be easily produced at room temperature without requiring thermal or chemical breakdown and uses environmentally friendly reaction solvents like ethanol and ultrapure water in place of the conventional solvents for free radical polymerization, such as toluene, chloroform, and acetonitrile. (Du et al. 2015).

Table 2.3. Comparison of various MIP preparation techniques and imprinting techniques.

Mechanism	Imprinting method	Advantage	Disadvantage
Free-radical polymerization	Bulk polymerization	Simplicity, High Purity, High Conversion Rates, Cost-Effective, Environmentally Friendly	Heat Management, Viscosity Increase, Difficulty in Controlling Polymer Properties, Risk of Gel Effect, Limited to Certain Monomers
	Solution Polymerization	Better Heat Management, Control over Molecular Weight, Ease of Handling, Versatility, Improved Polymer Properties	Solvent Removal, Environmental and Safety Concerns, Lower Polymer Purity, Solvent Compatibility, Potential for Solvent Effects
	Suspension Polymerization	Heat Management, Control over Particle Size, High Purity of Polymer Beads, Ease of Handling, Low Viscosity	Stabilizer Requirement, Complex Equipment, otential for Agglomeration, Limited to Hydrophobic Monomers, Environmental Concerns
	Emulsion Polymerization	Heat Management, High Molecular Weight Polymers, High Reaction Rates, Particle Size Control,	Residual Surfactants and Initiators, Process Complexity, Environmental Concerns, Energy-

		Low Viscosity, Versatile Applications	Intensive, Monomer Limitation
	Precipitation polymerization	Control over Particle Size and Shape, High Purity, Simple Separation, Reduced Viscosity Issues, Scalability	Limited Monomer Solubility, Solvent Recovery, Potential for Particle Aggregation, Environmental Concerns, Control of Reaction Conditions
Sol–gel process	Sol–gel	Low Processing Temperature, Homogeneous Mixing, Control over Material Properties, Versatility, High Purity and Uniformity, Fine Pore Structure	Long Processing Time, Shrinkage and Cracking, Complexity, Cost, Limited Scale-Up

2.4.4. Characterization methods

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have historically been used to study the morphologies of MIPs. Several fluorescence methods and atomic force microscopy (AFM) have also been beneficial in the characterisation of thin-film MIPs. In fact, characterizing monomer–template interactions using nuclear magnetic resonance (NMR), infrared (IR), and ultraviolet–visible spectroscopies is becoming a crucial part of molecularly imprinted polymer (MIP) design. This is true for both screening monomers for interaction with the template and validating computational design data.

Furthermore, a new trend appears to be implied by the notable rise in the quantity of spectroscopic investigations of ligand-MIP interactions. The application of diffraction investigations, X-ray photoelectron spectroscopy (XPS), and X-ray absorption fine structures has grown in popularity (Whitcombe et al. 2014). Nitrogen adsorption tests can be used to evaluate the specific surface areas and pore diameters of the polymers by Brunauer-Emmett-Teller (BET) analysis. Thermogravimetric analysis (TGA) is used to evaluate thermal stability. A vibrating sample magnetometer (VSM) is used to investigate the magnetic characteristics of materials that are magnetic, such as Fe_3O_4.

Table 2.4. Common characterisation techniques and their primary goals for MIPs

Physical Characterization	**Chemical Characterization**	**Thermal Characterization**	**Functional Characterization**
Scanning Electron Microscopy (SEM)	Fourier Transform Infrared Spectroscopy (FTIR)	Differential Scanning Calorimetry (DSC)	Binding Capacity and Selectivity Tests
Transmission Electron Microscopy (TEM)	Nuclear Magnetic Resonance (NMR) Spectroscopy	Thermogravimetric Analysis (TGA)	Rebinding Experiments
Atomic Force Microscopy (AFM)	X-ray Photoelectron Spectroscopy (XPS)		Kinetic Studies

3. MATERIALS AND METHODS

3.1. Materials

All the chemicals needed for this thesis study, i.e. L-Proline, L-Histidine, L-Phenylalanine, 1-Vinylimidazole (VIM), hydroxyethyl methacrylate (HEMA), Ethylene glycol dimethacrylate (EGDMA), Azobisisobutyronitrile (AIBN), Phosphate Buffer Saline (PBS), and ethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). The required solutions were obtained using Millipore Direct-Q3V Ultrapure water purification system (MerckAG, Darm-stadt, Germany) at room temperature and analytical-grade reagents. All chemicals were kept according to the directions, and waste was disposed of in accordance with the requirements. All of the chemicals used in our research fulfill reagent purity criteria.

3.2. Pro-MIP and NIP SPR Sensor Preparation

3.2.1. Surface Modification of SPR Gold Chip

Five milliliters of acidic piranha solution, a 1:1:3 mixture of hydrogen peroxide (H_2O_2), ammonia (NH_4OH), and water, were used to clean the SPR chip. The chip was then left on a heater for 10 minutes. After being taken out of the solution, the SPR chip was cleaned with ultrapure water and ethyl alcohol and dried for 15 minutes at 40°C in a vacuum oven. To modify the SPR chip, 10 µL of allyl mercaptan was added, covered, and overnighted in the fume hood.

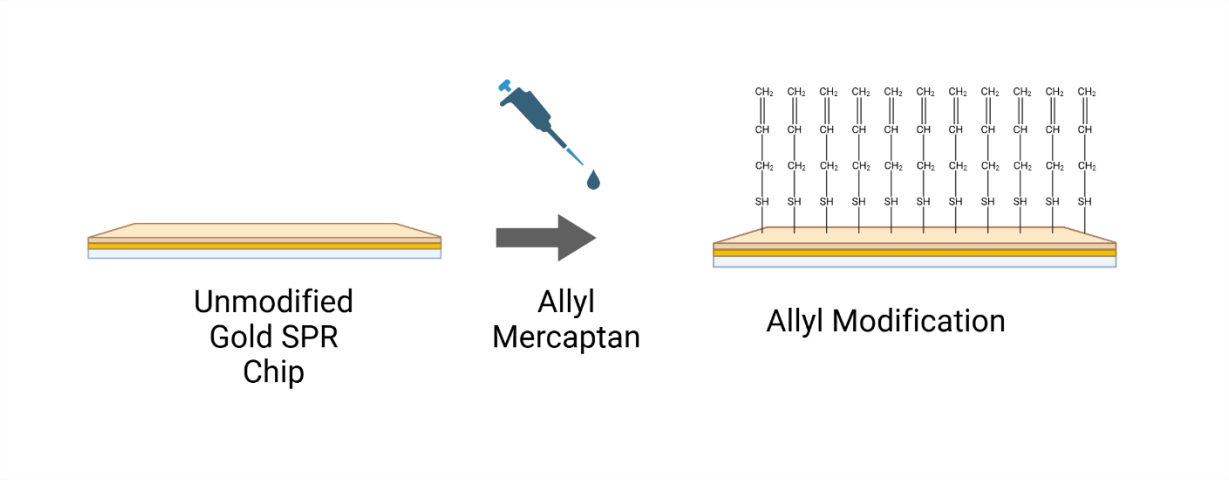

Figure 3.1. Surface Modification of SPR Chip (Created in https://BioRender.com).

3.2.2. Preparation of Pro-VIM Pre-complex

In order to form the Pro:VIM (1:4) pre-complex at the ideal molar ratio determined in the study by Nergiz et al., 4.6 mg Pro, 16 µL of 1-Vinyl imidazole and 400 µL of distilled water were added and mixed in eppendorf. The solution was kept for 4 hours at 4°C wrapped in aluminum foil to avoid being affected by the light.

3.2.3. Pro-MIP and NIP SPR Nanofilm Preparation

The Pro-VIM pre-complex was polymerized using the crosslinker EGDMA and the monomer HEMA to create Pro-MIP SPR chips. To the prepared precomplex, (20 µL) EGDMA and (40 µL) HEMA were added and mixed. 2.5 mg of prepared AIBN was added to the solution as an initiator, and it was well mixed until it was uniform. After dripping the solution (20 µL) onto the chip, it was spun in the spin coater device until the entire chip surface was covered. The produced SPR chips were exposed to a UV lamb (100 W, 356 nm, high intensity UV lamp) for 45 minutes in order to undergo the photopolymerization reaction. Without adding Pro to the polymer solution, the NIP SPR chips were made in the same manner.

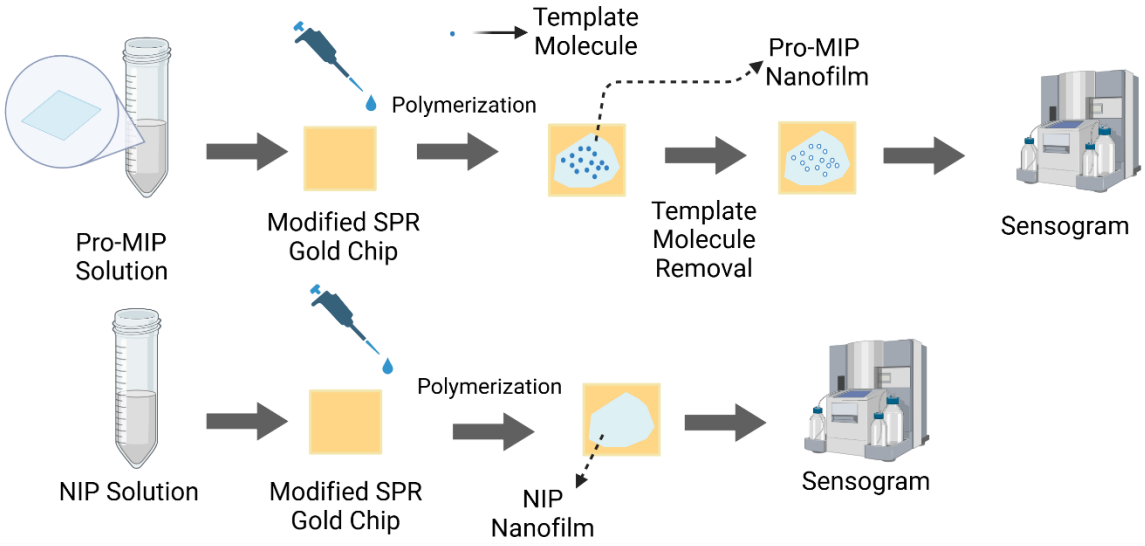

Figure 3.2. Pro-MIP and NIP SPR Nanofilms (Created in https://BioRender.com).

3.2.4. Template Removal Studies

A 0.03 M NaCl solution was used to separate the template molecule Pro from the SPR chips. After washing with a 0.03 M NaCl solution, the Pro-MIP SPR chip was placed. Desorption continued until the ΔR value was nearly zero.

3.3. Characterization of SPR Chips

3.3.1. SEM Analysis

SEM analysis is used to analyze the surface morphology of the produced nanofilm. Using a spin coater, 20 µl of the polymer solution was first put onto the SPR chip and spinned. Using a UV lamb, it was polymerized. SEM analysis was performed on the nanofilm that synthesized on the SPR chip. The gold-coated SPR chip was put on the instrument plate for SEM analysis. At Çukurova University Central Laboratory (ÇÜMERLAB), the SEM (Quanta 650 Field Emission SEM, FEI) was used for the SPR chips. The same magnifications (200000x) were used to acquire images from Pro-MIP and NIP SPR chips.

Figure 3.3. Scanning Electron Microscope (SEM)

3.3.2. FTIR Analysis

The chemical structures of the synthesized Pro-MIP and NIP nanofilms will be analyzed by FTIR. MIP and NIP nanofilms have been produced and added to the device in powder form for FTIR examination. To eliminate CO_2 and moisture from the measured sample chamber, N_2 gas was supplied for ten minutes. The wavenumber range (400–4000 cm^{-1}) was scanned to record the resulting spectra. FTIR analyses were performed at Hacettepe University Advanced Technologies Application and Research Center (Hünitek). Thermo ScientificTM (NicoletTM iS50) FTIR spectrophotometer was used.

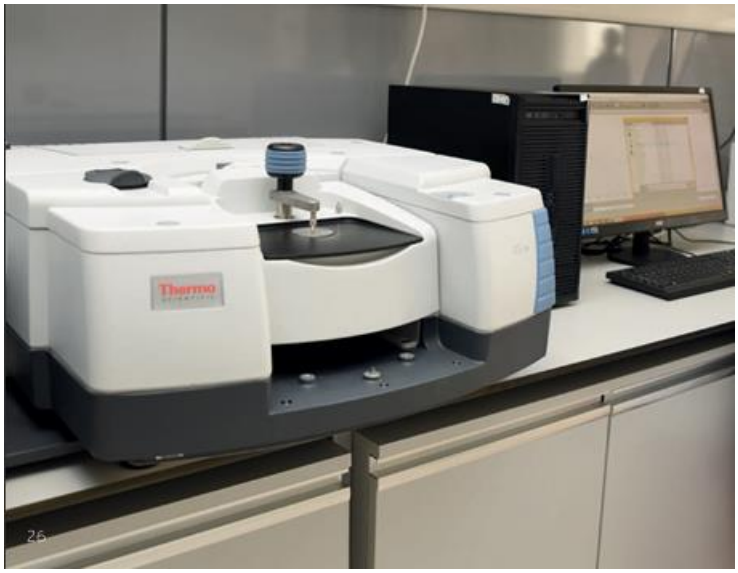

Figure 3.4. Fourier transform infrared spectrophotometer (FTIR)

3.3.3. Atomic Force Microscopy (AFM)

SPR chip atomic force characterization captured with a microscope in semi-contact mode. AFM was performed at Hacettepe University Advanced Technologies Application and Research Center (Hünitek). The AFM can conduct measurements at a very high resolution (4096 x 4096 pixels) because of its capabilities as a free cantilever interferometer. Double-sided adhesive tape was used to secure the SPR chip to the sample holder. Semi-contact imaging tests were conducted in the air. The frequency of vibrational resonance (341.30 kHz) was used. One VRMS is the vibration amplitude, and two VRMS is the empty vibration amplitude. The samples were obtained as 2 x 2 µm pictures with a resolution of 256 x 256 pixels and a scanning speed of 2 µm/s.

3.3.4. Contact Angle Measurements

The contact angle was measured using a device made by Biolin Scientific called the Attension Theta. Contact angle measurements were performed at Hacettepe University Advanced Technologies Application and Research Center (Hünitek). The instrument camera speed is 3009 FPS. The calculations use 1984 x 1264 pixel drop images, which are critical for measurement

accuracy. The contact angle was measured by dropping a drop of water onto the SPR chip using the sessile drop method. The contact angle of each image was measured independently after water was sprayed on different parts of the gold surfaces. The drop's left contact angle with the solid was measured at that location, and its right contact angle was recorded at that location. The mean of the two sites was used to determine the average contact angle values.

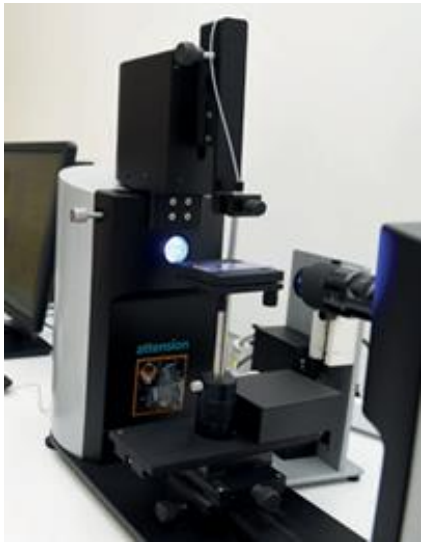

Figure 3.5. Contact angle device

3.4. Kinetic Analysis with Pro-MIP and NIP SPR Chips

Using Pro as the template molecule and VIM as the functional monomer in the Pro-VIM precomplex, the Pro-MIP SPR chip was prepared. Following the preparation of the Pro-MIP SPR chip, kinetic analyses were carried out utilizing Pro solutions at various concentrations. Prior to obtaining a steady peak, the prepared chip was put inside the apparatus and cleaned with ultrapure water (30 µl/min). Next, different concentrations of Pro solutions in water (0.5, 0.75, 1, 2.5, and 3.5 µg/mL) were made and added to the system one at a time. At a flow rate of 15 µL/min, it was used for 20 minutes. Pro binding caused a shift in resonant frequency at each concentration. A 0.03 M NaCl solution was used for desorption after the equilibrium state was achieved. Following the desorption process, ethyl alcohol and ultrapure water were used to wash the SPR chip.

3.5. Selectivity studies

The Pro-MIP of the SPR chip was placed into the SPR. After that, it was given a 10 minute water wash. At the same concentration (2,5 µg/mL), solutions of the Pro and competitor molecules (histidine and phenylalanine) were made in water and fed into the system separately, one after the other for 20 minutes (15 µl/min). When the equilibrium state was reached, desorption was performed for 10 minutes (30 µL/min) using a 0.03 M NaCl solution. After that, ultrapure water was used to wash the SPR chip once again.

3.6. Reusability

The reusability of Pro-MIP SPR chips was examined by feeding the SPR system five times with Pro solutions. After adding Pro solution to the SPR system sensorgrams were captured and the SPR chips effectiveness was determined.

3.7. Kinetic Analyzes From Human Serum

Selective Pro binding from human serum was also performed in human serum diluted 1:20000-fold with PBS buffer (10 µmM, pH 7.4). In this regard, samples from human serum (purchased from Sigma) were placed in EDTA-containing tubes and centrifuged at 3000 rpm for 30 minutes at room temperature to separate cells, filtered (3 µm Sartorius filter), and stored at -20 ˚C until use. Human plasma was melted at room temperature before use. Diluted plasma samples spiked with different concentrations of Pro (0.5, 0.75, 1, 2.5 µg/mL) were injected into the SPR system and sensorgrams were recorded. The same procedures used for the previous aqueous solution studies were used for the plasma studies.

4. RESULTS AND DISCUSSIONS

4.1. Producing Pro-MIP and NIP SPR Chips

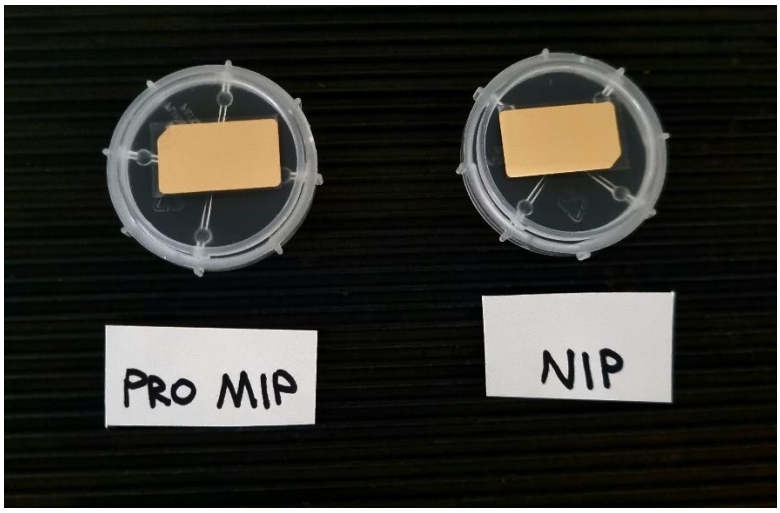

Figure 4.1. Pro-MIP and NIP SPR Chips

4.2. Characterization of Pro-MIP and NIP SPR Chips

4.2.1. SEM Images

Images from scanning electron microscopy (SEM) were used to visualize the surface morphology of the Pro-MIP and NIP SPR chips. On the surface, polymerization happened uniformly, as seen in Figure 4.2 Pro-MIP and NIP SPR chips SEM images were examined at a magnification of 200000X.

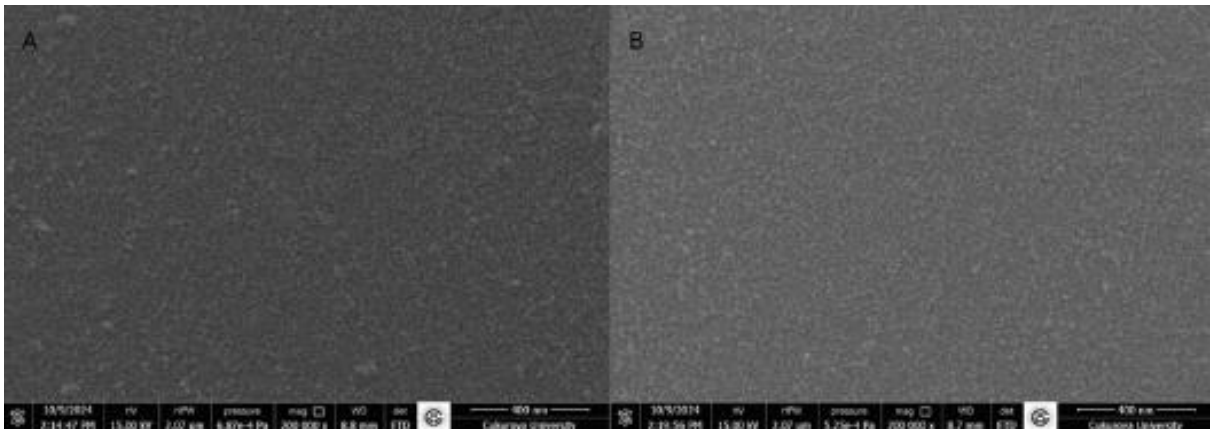

Figure 4.2. SEM images of SPR chips (200000X) A) Pro-MIP, B) NIP

4.2.2. FTIR Analysis

Upon analyzing the FTIR spectra of Pro-MIP and NIP it is seen that the spectra are very similar to each other, as expected. It's because the structure of the polymers forming both nanofilm structures is very similar. Some peaks observed in both spectra confirm the presence of HEMA and EGDMA components. Of these, the peaks observed around 2900 cm^{-1} represent aliphatic C-H stretching bonds and are thought to originate from aliphatic bonds in the structure of HEMA and EGDMA used. The C=O (carbonyl group) stretching vibrations, especially seen in the 1733 cm^{-1} region, indicate the ester groups of these components. These peaks consist of the vibrations of the ester groups of HEMA and EGDMA. The peaks observed around 1450 cm^{-1} represent CH_2 bending vibrations. At lower wavenumbers, peaks originating from external plane C-H bending vibrations are found around 900 cm^{-1} and 760 cm^{-1}. These peaks indicate the presence of a cross-linked polymer backbone.

However, significant differences were observed between the two spectra. The peak observed at 2960.9 cm^{-1} in the spectrum of NIP shifted to 2962.93 cm^{-1} in the spectrum of Pro-MIP and intensified. It is thought that this change observed in the graphs is due to the C-H stretching vibrations of the vinyl group in the VIM structure and the aliphatic C-H stretching vibrations in the Pro structure. In addition, two peaks are observed in the bands at 1376.65 cm^{-1} and 2853.53 cm^{-1}, which are seen in the spectrum of Pro-MIP but not in the spectrum of NIP. When these peaks are examined, the 1376.65 cm^{-1} peak is particularly associated with C-H bending vibrations. It is thought that the symmetric bending vibrations of the CH_3 methyl groups in Pro create a signal in this region. The cyclic amine structure and methyl groups in the Pro structure are effective in the emergence of this peak intensity. At the same time, the C-H bends in the side chain of the imidazole ring in the VIM structure may also contribute to this region. The 2853.53 cm^{-1} peak corresponds to the C-H stretching vibrations in the aliphatic structure. The C-H stretching of the aliphatic chains in Pro are observed in this region. The aliphatic C-H bonds in the cyclic structure of Pro create a peak around 2850 cm^{-1}. In addition, the C-H stretching to which the sp^2 hybridized carbons of the vinyl group in VIM are attached also affect this region and are reflected in the spectrum. That is, the peak at 1376.65 cm^{-1} indicates the C-H bending vibrations of the methyl groups in Pro and the bending around the imidazole ring of VIM, while the peak at 2853.53 cm^{-1} represents the C-H stretching vibrations of the aliphatic chains of Pro and the vinyl group of VIM.

In conclusion, all these changes observed in the FTIR spectrum of Pro-MIP confirm the presence of the Pro:VIM pre-complex in the chemical structure of the polymer.

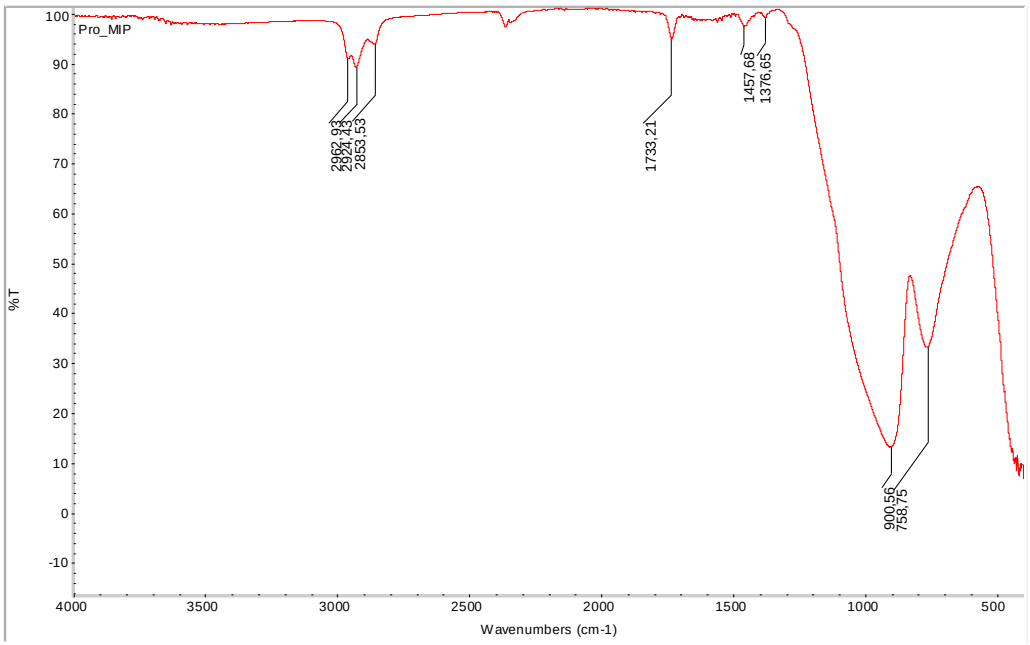

Figure 4.3. The FTIR spectrum of Pro-MIP

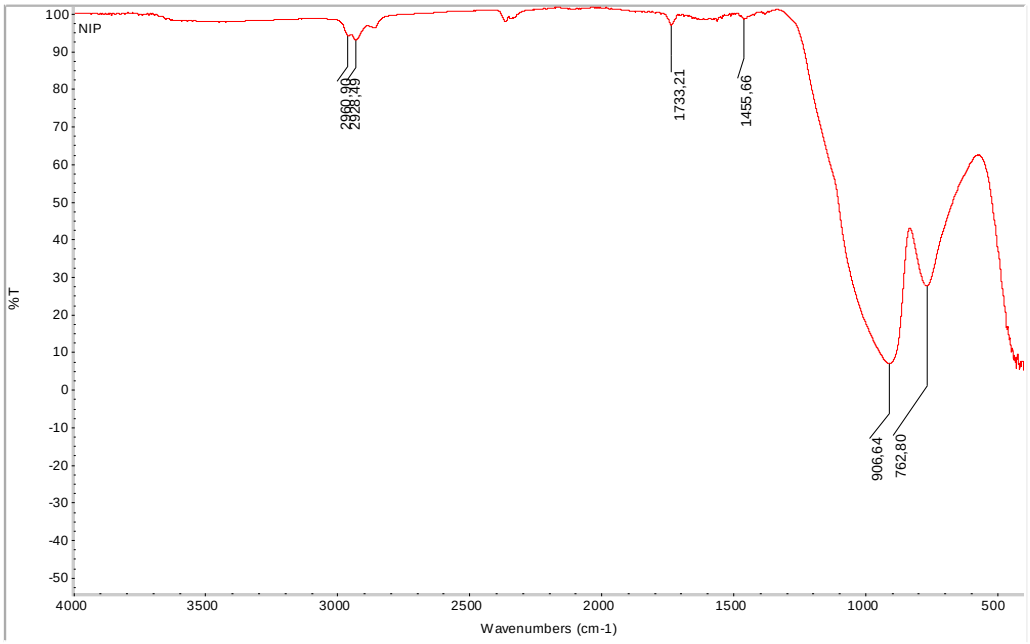

Figure 4.4. The FTIR spectrum of NIP

4.2.3. Atomic Force Microscopy (AFM)

Atomic force microscopy was used in half-contact mode to analyze the surface morphology of the NIP and Pro-MIP SPR chips. The Pro-MIP SPR chip's surface depth was estimated to be approximately 170 nm, while the surface depth of the NIP chip was 160 nm.

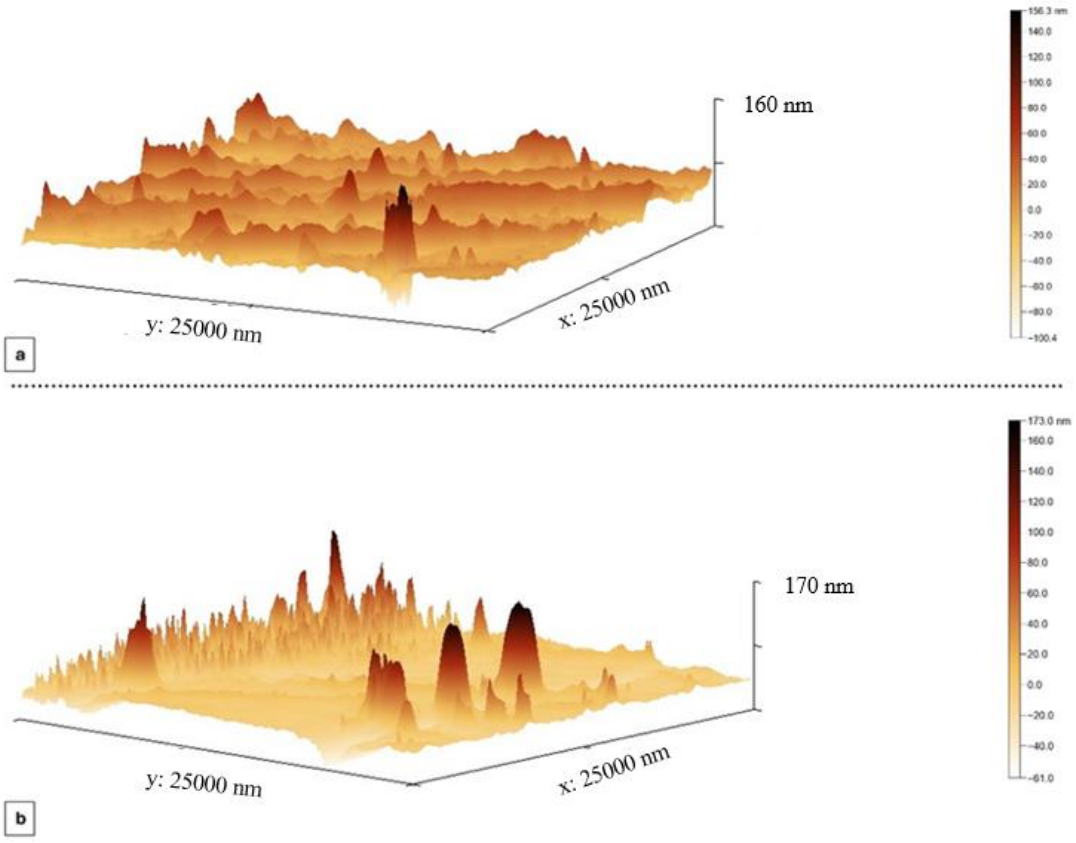

Figure 4.5. AFM Images (a) NIP chip, (b) Pro-MIP SPR chip

4.2.4. Contact Angle Measurements

The Attension Theta, a device made by Biolin Scientific, was used to characterize the surfaces of the SPR, Pro-MIP, and NIP SPR chips. Water is first dripped over the chip once it has been positioned on the apparatus with its nanofilm-coated surface facing up. The contact angle value is determined by averaging the several measurements that are obtained from the right and left. When water comes into contact with a solid surface, a specific amount of angle is created. This angle indicates the degree of wettability and varies according to the solid's contact with the contacting liquid. The cohesion and adhesion forces determine the contact angle's size. The Pro-MIP and NIP SPR chips surface contact angles are shown in Figure 4.6.

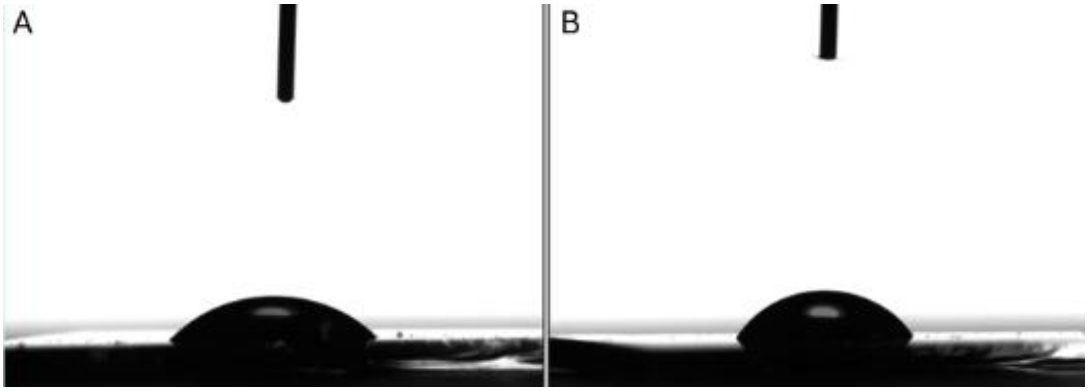

Figure 4.6. Contact angle image of SPR chips. A) Pro-MIP , B) NIP

Table 4.1. The contact angles of surfaces

Surface	**Contact Angle, º**
MIP SPR chip	48,62
NIP SPR chip	58,84

Contact angle measurements show that after the Pro was imprinted, the NIP SPR chip surface's contact angle value of 58,84 dropped to 48,62. The hydrophilic property of the surface rises when the contact angle of the surface significantly decreases. This circumstance can be

explained by the fact that HEMA, a functional monomer with a very hydrophilic nature, was utilized to create the surfaces of both MIP and NIP nanofilms. Therefore, the hydrophilicity of the surface grew and the contact angle value reduced when a hydrophilic polymer was bound to the surface. The surface becomes rougher due to the formation of MIP and NIP nanofilm layers, and the surface becomes more hydrophilic as a result of the increased surface roughness. The Pro-MIP SPR chip has more surface roughness than the NIP SPR chip due to its VIM and Pro presence. Consequently, compared to the NIP SPR chip, the contact angle is decreased significantly.

4.3. Kinetic Analysis

4.3.1. Kinetic Analysis with Pro-MIP SPR Chips

A Pro-MIP SPR chip was made for this investigation. Pro solutions were made at various concentrations (0.5, 0.75, 1, 2.5, 3.5 µg/mL) in order to examine the connection between the Pro concentration and the SPR signal. The SPRview program was used to gather the kinetic data after the solutions interacted with the SPR. Figure 4.7 displays the sensorgrams produced with various Pro solution concentrations.

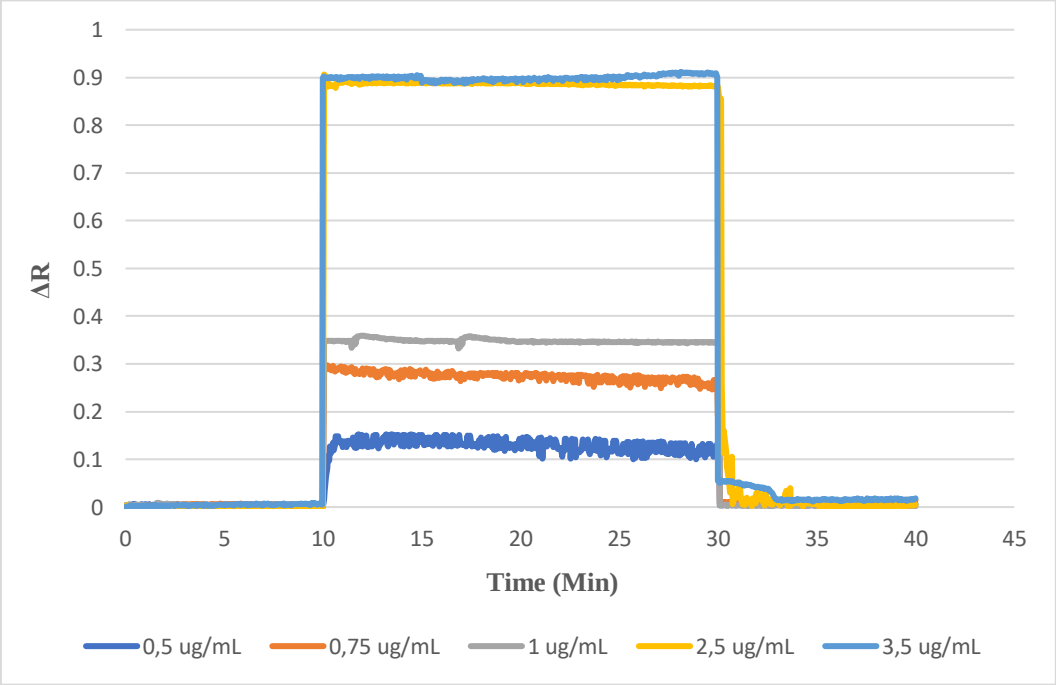

Figure 4.7. Sensograms obtained with different concentrations of Pro solutions

The sensogram graph in Figure 4.7 shows the time-dependent change in the signal ΔR as the Pro molecule moves through the system.

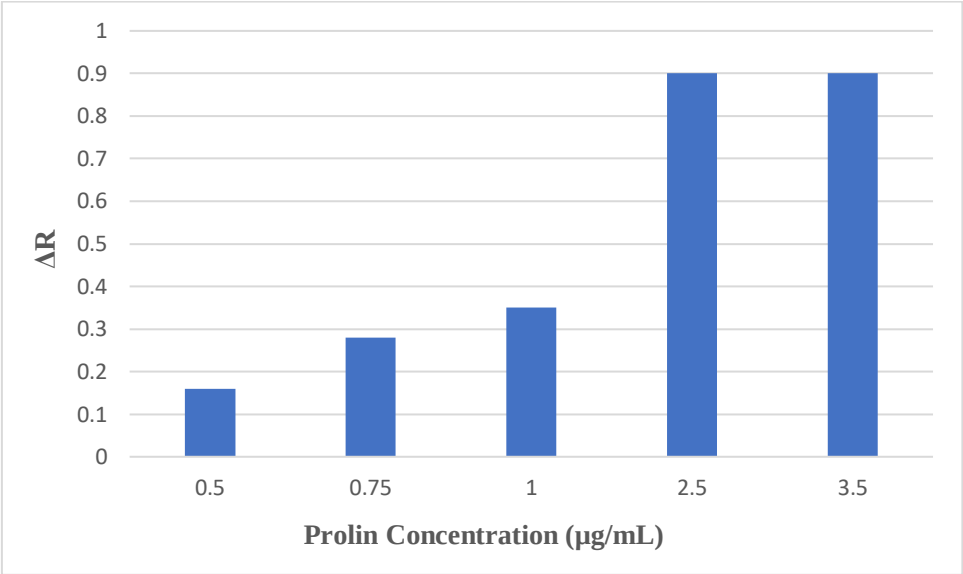

Figure 4.8. The relationship between the Pro-MIP SPR chip's ΔR and Pro concentration

4.3.2. Adsorption Isotherm Models

The adsorption capacity is calculated using three distinct models.

1- Freundlich Model

The value of surface heterogeneity is calculated using the Freundlich model. It is assumed that many adsorption layers form in order for adhesion to occur.

$\Delta R = \Delta Rmax\ [C]\ 1/n$

The heterogeneity index (1/n) changes between 0 and 1 in the model that was computed using the equation. The homogeneity increases as the heterogeneity index approaches 1. We can state that the system is homogeneous when the value is 1.

2- Langmuir Model

The value of surface homogeneity can be determined using the Langmuir model. It makes the assumption that molecules adhere to a certain number of distinct surfaces, each of which has a capacity of only one molecule.

$\Delta R = (\Delta RmaxC)/(KD+[C])$

3- Freundlich - Langmuir Model

In settings where Freundlich and Langmuir models are suitable, the Langmuir-Freundlich model can be used. Interactions on both homogeneous and heterogeneous surfaces are explained by this model.

$\Delta R = \Delta Rmax[C]1/n / KD+[C]1/n$

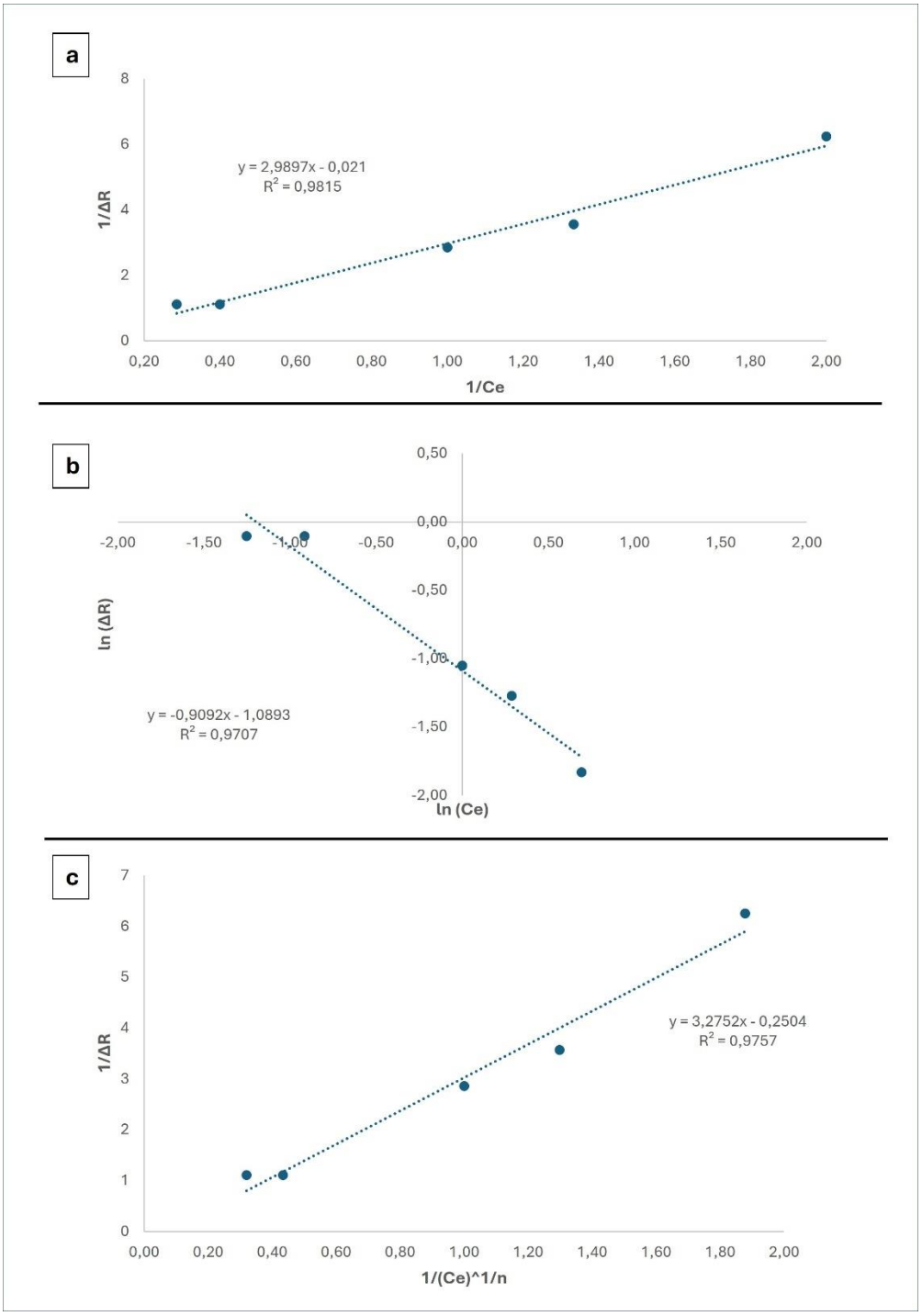

Figure 4.9. Adsorption models. a) Langmuir, b) Freundlich, c) Langmuir - Freundlich.

Table 4.2. Langmuir, Freundlich and Langmuir-Freundlich parameters

	Langmuir	**Freundlich**	**Langmuir-Freundlich**
ΔRmax (µg/mL)	47,6190	2,9722	3,9936
KD (mL/ µg)	142,3667	-	13,08
KA (µg /mL)	0,0070	-	0,0765
R^2	0,98	0,97	0,97
1/n	-	0,9092	0,9092

Figure 4.9. illustrates how the experimentally obtained data mostly agree with the Langmuir model (R^2 = 0.98). This implies that the Pro binding sites on the prepared imprinted sensor surface are single-layered, consistently spaced, energy-equal, and have minimal lateral interaction. The three isotherm models' results are shown in Table 4.2.

4.4. Selectivity Studies

The ability to directly detect the target molecule without the need for a labeling step is the most significant characteristic of molecularly imprinted polymer-based SPR chips. As a result, the sensor's selectivity and the quality of the produced molecular imprinting are closely correlated. The imprinting selectivity coefficients and the selectivity coefficient are used to determine the prepared molecularly imprinted sensors' selectivity.

The following formula is used to get the selectivity coefficient.

k= ΔRtemplate / ΔRcompetitor

The nanofilm-based SPR sensor's selectivity was investigated using MIP and NIP SPR chips. For selectivity control, Histidine and Phenylalanine were used as competing molecules in contrast to the target molecule Pro. Three distinct compounds at a concentration of 2,5 µg/mL were run through the SPR device.

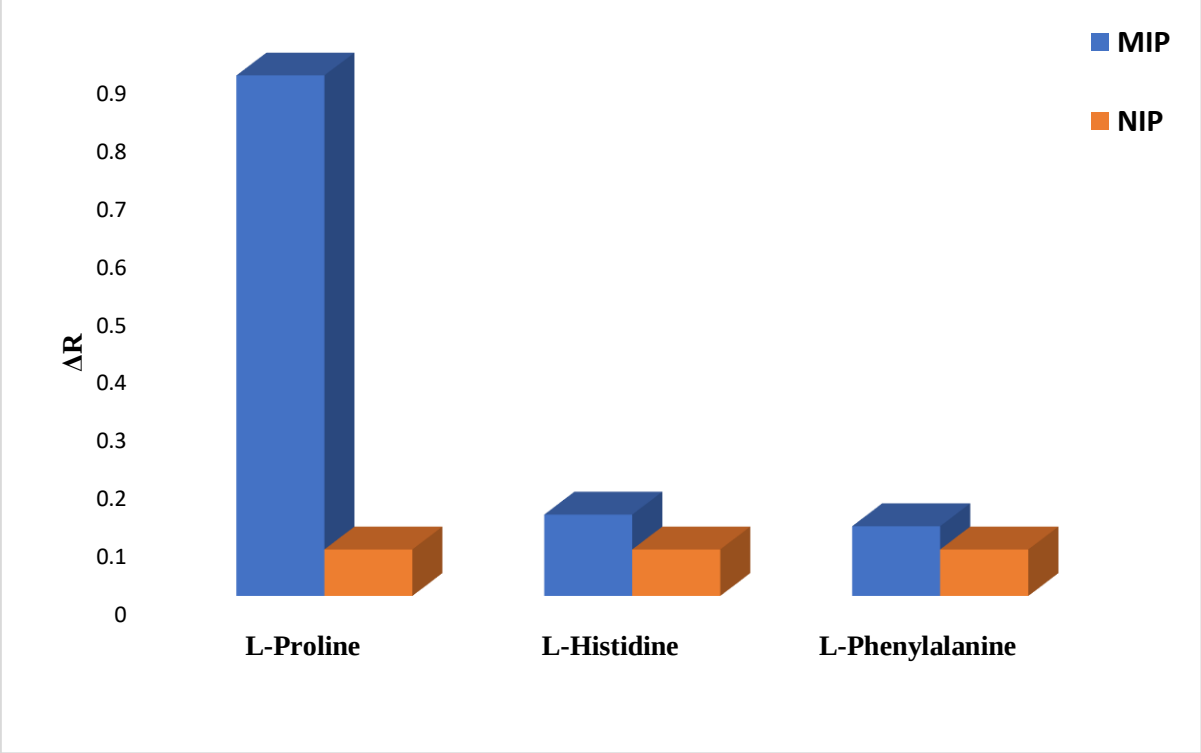

Figure 4.10. ΔR values of Pro, Histidine and Phenylalanine competitor molecules (2,5 µg/mL) for Pro-MIP and NIP SPR chip interactions.

Table 4.3. Pro's selectivity and relative selectivity coefficients in relation to phenylalanine and histidine

Competitor Molecules	MIP		NIP	
	ΔR	k	ΔR	k
L-Proline	0,900	-	0,080	-
L-Histidine	0,140	6,429	0,080	1,000
L-Phenylalanine	0,120	7,500	0,080	1,000

4.5. Reusability Studies

The sensogram produced by applying Pro solution to the sensor surface five times in a row is displayed in the figure to track the Pro-MIP SPR chip's reuse. The solution used for regeneration was 0.03 M NaCl. Five repeated additions of Pro solution to the system were found to be reusable without causing any discernible changes to the binding sites.

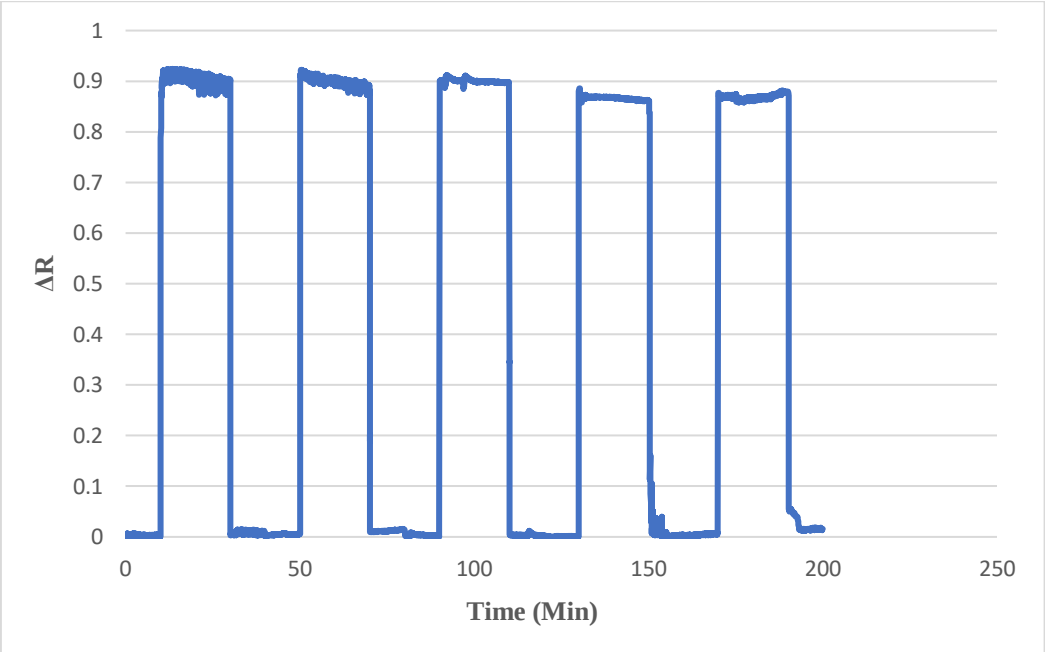

Figure 4.11. Reusability of SPR chip

4.6. Kinetic Analyzes From Human Serum

Pro was found in human serum samples using a Pro-MIP SPR device. Pro concentrations of 0.5 µg/mL to 2.5 µg/mL were added to plasma samples that had been diluted by 1/20000. A shift in peaks was noted in the analyses carried out using the Pro-MIP SPR chip in correlation with the rise in Pro concentration. Figure 4.12 displays these samples' sensogram curve.

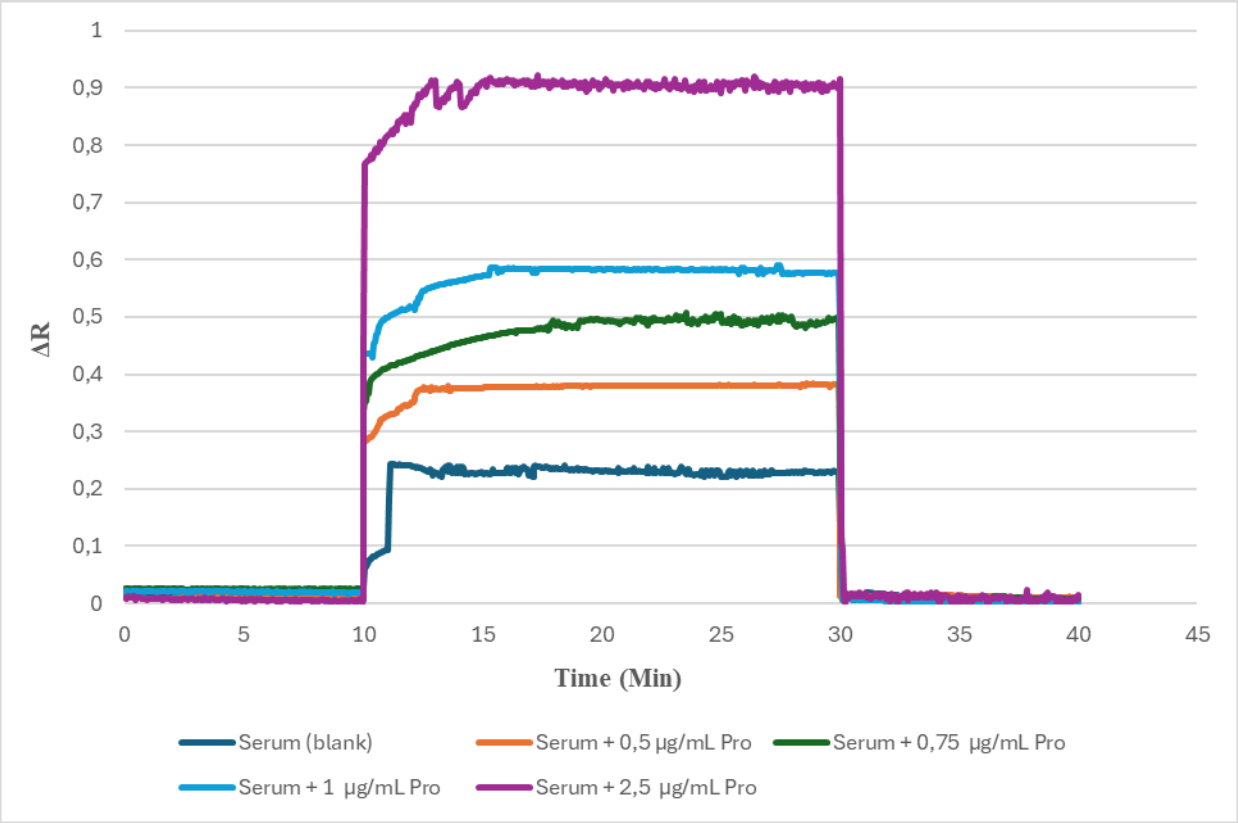

Figure 4.12. Sensograms of interactions between plasma and SPR chip.

Sensograms showing time-varying peaks from plasma studies are shown in Figure 4.12. The graphic illustrates how the Pro-MIP SPR chip's signal value rises as concentration does. The SPR chip seems to signal even at a very high dilution rate (20000 times). The most significant finding from the tests conducted in this context is that Pro can be found even in complicated environments like plasma, which is composed of 91% water, 7% proteins, 2% ions, nutrients, gasses, wastes, and hormones. Therefore, Pro may be efficiently determined from both natural sources and aqueous solutions using Pro-MIP SPR chips.

CONCLUSION

This thesis aimed to develop L-Proline imprinted nanofilm coated SPR biosensors that offer high selectivity and sensitivity for the diagnosis and monitoring of esophageal cancer and hyperprolinemia.

These innovative sensors, which have no equivalent in the literature, enabled the specific identification of Pro in human serum at low detection limits like 0.5 µg/mL. Pro-MIP SPR sensors were successfully validated in the characterization analyses (SEM, FTIR, AFM and contact angle measurements). In particular, the unique changes observed in the FTIR spectra supported the existence of the Pro:VIM pre-complex and the correct polymerization. In addition, kinetic analyses and adsorption isotherm models revealed that Pro-MIP SPR sensors fit the Langmuir model and showed high selectivity against L-Proline. These sensors provided 6.4 and 7.5 times higher selectivity compared to competitor molecules such as histidine and phenylalanine, respectively. The capability of Pro-MIP SPR sensors to detect Pro from real human serum has been successfully evaluated. Reusability studies have shown that Pro-MIP SPR sensors can be used five times without a significant decrease in adsorption capacity. This feature shows that the sensors offer a significant advantage in terms of cost-effectiveness and practical use. Kinetic analyses performed with human serum samples have shown that the sensor detects Pro molecules even at low concentrations with high sensitivity. In particular, the detection limit of 0.5 µg/mL has revealed that the sensor exhibits superior performance in biomarker analysis in human serum. In addition, sensograms obtained from serum samples emphasize that the sensor produces consistent results in terms of selectivity and accuracy. These results show that the developed Pro-MIP SPR biosensors are promising not only for use in laboratory environments but also in real clinical applications.

As a result, the developed Pro-MIP SPR biosensors stand out as an innovative technology that offers high sensitivity, low detection limit and rapid analysis compared to traditional methods for biomarker detection. It has the potential to be used in various areas such as clinical diagnosis and monitoring of metabolic diseases and will pave the way for other studies in this area. Future studies will be able to take this technology to further levels by investigating the adaptability of sensors for different biomarkers and wider application areas.

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