Universidad de Valladolid

# A chitosan-based molecularly imprinted polymer sensor modified with nanoparticles for sugar detection


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## Summary

The use of sensors in the food industry is essential to determine and preserve the quality of their products. In the food industry, the detection, analysis, quantification, and evaluation of sugars are vital for ensuring and guaranteeing the quality and security of food and beverages. Therefore, a wide variety of electrochemical sensors based on the molecularly imprinted polymer (MIP) technique have caught a lot of attention in detecting sugar. After the design of these sensors, the response can be measured by potentiometric or cyclic voltammetric methods.

This investigation uses chitosan as a biopolymer to develop MIP sensors. These sensors detect sugars like glucose and lactose. The MIP layer is deposited in a glassy carbon electrode (GCE). The response of the MIP sensor is evaluated with a non-imprinted polymer (NIP) sensor created by the absence of the templated molecule.

To improve the sensor's capabilities, the influence of adding nanoparticles in the development of MIP sensors has been evaluated. Metallic nanoparticles like gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) were used. On the influence of AgNPs, the MIP sensor has a much higher response than the non-modified sensor.

The optimized MIP sensor is a viable analytical method for food industrial applications. It detects the presence of sugar in food and raw materials used in food processing.

## Keywords

Electrochemical sensor, MIP, potentiometry, cyclic voltammetry, chitosan, AuNPs, AgNPs, glucose, lactose.

# Impact on the Sustainable Development Goals

The UN's Sustainable Development Goals are 17 interlinked goals that set a blueprint for sustainable economic growth, created and approved in 2015 to achieve in 2030. Designed for sustained growth, the SDGs aim to respond to global concerns like poverty, inequality, climate change, environmental degradation, peace, and justice.

The goals are the following:

Goal 1: No Poverty

Goal 2: Zero Hunger

Goal 3: Good Health and Well-Being

Goal 4: Quality Education

Goal 5: Gender Equality

Goal 6: Clean Water and Sanitation

Goal 7: Affordable and Clean Energy

Goal 8. Decent Work and Economic Growth

Goal 9: Industry, Innovation, and Infrastructure

Goal 10: Reduced Inequalities

Goal 11: Sustainable Cities and Communities

Goal 12: Responsible Consumption and Production

Goal 13: Climate Action

Goal 14: Life Below Water

Goal 15: Life on Land

Goal 16: Peace, Justice, and a Strong Institution

Goal 17: Partnerships

From the perspective of this goal, goal 9 has directly impacted this work in the thesis. Goal 9 is the upbringing of industries with good innovation and infrastructure. Developing innovative sensors with industrial capabilities will ensure the fulfillment of this goal. Also, this investigation indirectly affects the goals 1, 2 and 3. Goal 3 is good health and well-being; food safety is essential to maintain good health and well-being. This investigation ensures it by developing the applicable sensor for the food industry. The growth in the industry reduces poverty. Therefore, 1 and 2 goals can be achieved.

# CHAPTER 1

# INTRODUCTION AND OBJECTIVE

# 1. INTRODUCTION AND OBJECTIVE

## 1.1 INTRODUCTION

The field of nanoscience revolves around the study of materials smaller than 100 nanometers, often referred to as nanomaterials.[1] These materials play a crucial role in developing nanodevices, which are vital in modern analytical science, a branch of chemistry that focuses on creating techniques and tools for identifying substance compositions and providing quantitative and qualitative properties of various organic and inorganic materials.[2] Incorporating nanomaterials in nanodevices has enhanced their analytical properties by modifying physical and chemical properties such as surface area and degree of functionalization.[3] This integration of nanomaterials into analytical devices, known as nano analytics, is divided into two main parts: the analysis and characterization of nanomaterials and the examination of nanomaterial-infused tools and devices, which can encompass the creation of nano-sensors and nanostructured electrodes.[4] As a result, the establishment of nanosensors holds significant importance in the domains of nanoscience and nanotechnology.

In the present world, among other components, sugar monitoring is critical. In the case of glucose detection, in recent studies, Golobly estimates that in the age group 20-79, the possibility of having diabetes in 2021 is around 540 million people. This amount rises by 12.2% in 2045, reaching 783.2 million people.[5] Another sugar necessary for detection is lactose due to the health concern that, according to lactose intolerance studies, nearly 75% of the world's population may lose the ability to convert lactose digestion. Lactose intolerance can manifest in two ways, depending on genetic variables. It can be lost during infancy and in maturity.[6] The management of illnesses like diabetes or lactose intolerance highlights the significance of developing devices able to detect compounds like glucose and lactose.[7] The conventional analytical method is still used to detect sugars but presents many disadvantages. To overcome these disadvantages, such as the high cost of production and extensive procedure time of conventional analytical methods, alternative techniques like the use of sensors have been implemented. Electrochemical sensors are an attractive choice among various sensors because they are susceptible and stable. Moreover, fabricating these types of sensors is more effective and inexpensive. These

advantages make electrochemical sensors theoretical notions and practical tools that play an essential role in the food industry.[8]

Electrochemical sensors are diverse and highly effective methods in modern analytics. Molecularly Imprinted Polymers (MIPs) are polymers that create an imprint of a particular molecule, known as a template molecule. This technique creates a cavity in the polymeric matrixes, which selectively binds only with the template molecule, which is determined in the production of MIPs. So, this technique is a synthetic adaptation of biological systems like antibody-antigen systems. Compared to biological receptors used in the biological sensor, MIPs offer high selectivity, specificity, and durability in sensor development. Also, it is less expensive than the development of a biological sensor.[9]

The investigation for this Master's Thesis was accomplished under the guidance of UVaSens researchers. This group of investigators is extremely proficient in preparing nanostructured sensors with techniques like Langmuir-Blodgett or layer-by-layer in electrochemical sensors and biosensors. The novelty of the developed sensors-based molecularly imprinted technique makes this investigation different from the rest of the previous research in the group.

This study mainly focused on developing different sensors based on molecularly imprinted polymer technology, incorporating metal nanomaterials to increase their sensing ability for detecting sugars such as glucose or lactose. These sensors were designed to detect sugars commonly present in food and beverages.

## 1.2. OBJECTIVE

This investigation aims to develop electrochemical nanosensors based on the molecular imprinting technique to detect glucose and lactose. It also evaluates the impact of metal nanoparticles (AgNPs, AuNPs) in MIP sensors. The objectives that must be fulfilled to obtain the final goal are,

1. Developing MIP sensors through electrodeposition in the presence of a template molecule.
2. Study the influence of elution and the type of eluents
3. Evaluation of electrochemical response compared with NIPs (Non-imprinted Polymers) and MIPs (Molecularly Imprinted Polymers) sensors.
4. The development and optimization of nanoparticle-modified MIP sensors and NIP sensors to improve their sensing properties.
5. Analysis and evaluation of sensors through potentiometric and cyclic voltammetric methods to detect glucose and lactose.

# CHAPTER 2

# REVIEW OF LITERATURE

# 2. REVIEW OF LITERATURE

## 2.1. Introduction of Nanoscience and Nanotechnology

In science, nanoscience combines physics, chemistry, biology, and engineering. Its definition is a technology that ensures the manipulation of matter at the level of atoms or molecules to build nanoscale products [10]. The main advances and studies are conducted on a 1 to 100-nanometer scale. In this scale, materials exhibit different physical, chemical, and biological properties of the same material in the larger microscope scale. It increases the novelty and unpredictability of nanoscale materials, even though the term “Nanoscience” was coined early in the 1970s. However, the 1980s, after the development of the Scanning Scope Microscope (STM), is considered the beginning of modern nanotechnology. The early discoveries and origin of present nanotechnology and nanoscience happened in the last two decades of the twentieth century. For example, the discovery of fullerenes in 1985 [11], and the discovery of carbon nanotubes in 1991 [12]. It is a significant finding because this discovery has many applications in electronics, material science, and biomedical engineering. Currently, most applications are done in sensory analysis, electronics, energy production, medicine, and the food industry.[13]

## 2.2. Chemical sensor

Chemical sensors are devices that detect and measure chemical changes and then convert the change into monitorable signals. Based on the signal output produced by the chemical sensors, the sensor is classified into six groups. Optical sensors, magnetic sensors and thermometric sensors, mass sensors, electrical sensors, and electrochemical sensors [14].

The optical sensor uses the optical properties of the indicator to analyse the chemical chemicals occurring in the sample. However, the magnetic sensor detects the change in the sample system's magnetism magnitude. Thermometric sensors measure changes in the temperature sample. Likewise, mass sensors measure changes in the mass due to the accumulation of the analyte.

The electrical sensor measures the change in the electrical signal by accumulation of analyte. But in, the electrochemical sensors measure the electrochemical changes in the electrode and the analyte [15].

Electrochemical sensors, a modern analytical technique, are pivotal in various fields, including physical chemistry, analytical chemistry, solid-state physics, biochemistry, and statistical analysis. They are crucial in quantitative and qualitative analysis of desired chemical species, followed by chemical signal analysis. The transducer, a vital component, converts chemical changes into analytical signals. Electrochemical sensors detect various media, including solid, liquid, or gaseous phases. Its usage has increased due to its low production cost, high sensitivity and selectivity, and the simplicity of its experimental methodology. Its wide detection range and versatility have made it a staple in the scientific community [16].

## 2.3. Electrochemical sensors.

The electrochemical sensors are classified into different types: Potentiometric sensors, which use the equilibrium potential of a working electrode and reference electrode at zero current, and Voltammetry sensors, where current measurements are taken from the redox activity of the analytes on the surface electrode. Conductometry sensors, where the electric conductivity of the sensor and analyte was measured; impedimetric sensors were used to calculate the change in the charge transfer resistance, and amperometric sensors worked under the change transfer rate at the electrode surface.[17]

This investigation mainly used readout technology like potentiometry or voltammetry techniques based on the novelty of these sensors in these specific techniques. The properties of the template molecules (glucose and lactose) used in the investigation also led to utilizing these techniques. Similar studies on glucose electrochemical sensors suggest that potentiometry or voltammetry readout techniques are being explored for their implementation.[18–20]

### 2.3.1. Potentiometry

In potentiometry, the equilibrium potential of a working electrode and reference electrode is used at zero current. Potentiostat classification is based on the applied voltage signal and corresponding current waveforms. This method monitors the potential between the reference and

counter-electrodes and compensates for the voltage between the reference and working electrodes.[21]. The principle of this method is based on converting the activity of electrodes ion activity into a potential according to the Nernst equation. The response-based Nernst equation is typically referred to as classical potentiometry. The integrated version of potentiometry of other electrochemical methods is referred to as non-classic potentiometry or dynamic potentiometry.[22] Therefore, a MIP-based potentiometric sensor is classified as a non-classic potentiometric. Typically, potentiometric methods are used to detect inorganic material. However, MIP-based potentiometric sensors detect biological species and organic materials.[23] The potentiometric technique follows a 2-electrode system setup, while the cyclic voltammetry technique consists of 3-electrode setups. A 2-electrode comprises one working electrode (WE) and one counter electrode (CE). In the 2-electrode system, the counter electrode also acts as a reference electrode. Figure 1 is a schematic representation of the 2-electrode system of potentiometric sensors.

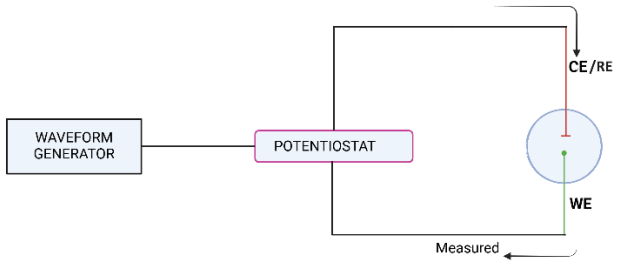


*Figure 1 Schematic representation of potentiometric response*

### 2.3.1.1. Potentiometric electrodes

In a 2-electrode potentiometric system, the potentiometric electrode is the working electrode. These potentiometric electrodes are electrochemical sensors that measure the solution's potential based on the concentration of specific ions. In potentiometric sensors, ion-selective electrodes are commonly used electrodes. The analytes dictate the usage of ion-selective electrodes (ISEs).

As a result, it possesses excellent selectivity for the analyte. The glass electrode used for measuring pH is a typical example of ISEs. The ion exchange process makes the glass membrane susceptible to hydrogen ions. Potentiometry also includes metal and membrane electrodes. Another type of electrode classification potentiometric sensor is membrane electrodes, used to test individual molecules, and metal electrodes, used to evaluate the reactions' analytes.[22,24] The metal electrodes are also known as solid-state electrodes.

### 2.3.2. Cyclic voltammetry

Cyclic voltammetry is an electrochemical process that studies the redox properties of chemical compounds. It gives information about chemical and physical changes in electrochemical reactions. The term cyclic voltammetry refers to the potential being cycled more than once. This technique's main advantage is suppressing the background current and enhancing the detection limit.[25] The voltammetric MIP sensor acts as a recognition element when selective binding occurs on the surface of the electrode, leading to a change in current based on potentials. As mentioned before, cycle voltammetry analyzes the sensor using a 3-electrode system. It consists of a working electrode (WE), a reference electrode (RE), and a counter electrode (CE). Figure 2 represents the 3-electrode system of the cycle voltammetry technique. In this system, the working electrode acts as the voltammetric sensor.

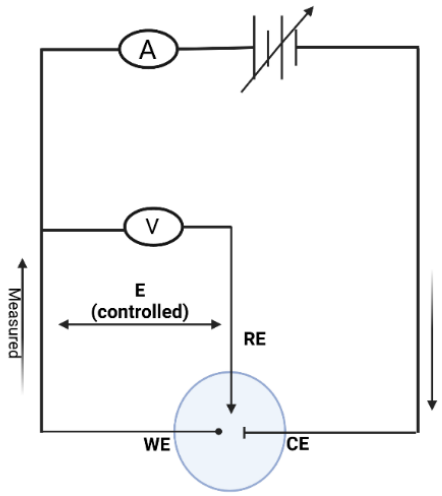


*Figure 2 Schematic representation of cyclic voltammetry setup.*

#### 2.3.2.1. Voltammetric Electrodes

Different electrochemical analysis methods use various types of electrodes. Silver, gold, and platinum are commonly used as voltammetric sensor electrodes. The main advantage of these novel metal electrodes is their inertness. These types of electrodes exhibit good conductivity and are suitable for voltammetric analysis because they do not intervene in the oxidation and reduction of the electrolytic cell. The drawback of these electrodes is surface oxidation. For this reason, the electrodes made of carbon can solve this problem and avoid the presence of impurities on the surface because they are easily removable. Therefore, carbon electrodes have replaced metal electrodes in recent years. The carbon exists in different allotropic forms. So, carbon electrodes are also presented in various forms. Graphite electrodes, glassy carbon electrodes, diamond-like carbon, etc., have been involved in various studies. For example, carbon-based biosensors are mainly used to detect drugs, proteins, and biomarkers because solid and flexible carbon materials show some level of biocompatibility with the biological media. [26] Among carbon-based electrodes, glassy carbon electrodes are widely used in electrochemical applications. The primary reason for using glassy carbon electrodes is that they are stable and compact. Another significance is that they provide a wide potential range with a minimum background current.[27] MIP sensors for detecting dopamine in biological samples were utilized for the advantages of glassy carbon electrodes (GCE).[28]

Different pretreatments are available to prepare the surface of glassy carbon electrodes for electrochemical analysis. Polishing with alumina and the use of emery paper is widely acceptable. Laser treatment and ultrasound have also been used to make a reproducible surface in glassy carbon electrodes.[29]

In recent advancements, materials like nanoparticles (NPs), carbon nanotubes (CNTs), quantum dots, graphene, etc., have been introduced into the surface of electrodes to improve their analytical capabilities.[30,31] The nanomaterials and their special optical, electronic, mechanical, and magnetic features will enhance the sensor's ability to be utilized in different electrochemical applications.

#### 2.3.2.2. Reference electrodes

The reference electrodes provide a stable and accurate potential compared to the working electrodes. These potential discrepancies lead to an examination of the working electrodes. Two types of reference electrodes are used for analytical purposes: Ag/AgCl and Hg(l)/Hg2Cl2. The elements need to have a specific chlorine concentration to function as reference electrodes. Thus, the components are submerged in the saturated KCl solution. [24,32]

#### 2.3.2.3. Counter electrodes

Counter electrodes, also known as auxiliary electrodes, play a vital role in the 3-electrode system. In this system, the counter electrode completes the electrical circuit by allowing electrons to flow through the counter and working electrodes. These electrodes are made of inert materials to prevent oxidation reactions in electrolytic cells. The widely used counter electrode in cyclic voltammetry is platinum, an inert metal with high conductivity and chemical stability to the electrochemical reaction. Some other counter electrodes, like carbon or graphite, have also been used, depending on the experimental conditions.[33,34]

### 2.3.1. Biosensors

The sensors are analytical devices classified based on various criteria; one classification is based on the transduction method, and another classification-based recognition element or physicochemical detector is used in the sensor. The biosensor uses biological components as a recognition element.

A biosensor is a device that gives quantitive analytical information when the sensor detects biological analyte with the help of the biological recognizer in the sensor. Typically, biological molecules like antibodies, tissues, cell receptors, enzymes, organelles, etc., are presented as bio-receptors in biosensors. The recognition process in the biosensor is assessed by a suitable tranducer that can recognize the physicochemical change that occurs in the sensor. The stability and reproducibility of the biosensor depend on the interaction between the bio-recognizer and the

transducer [35]. Due to materials science and nanotechnology advances, electrochemical sensors have recently incorporated new methods to enhance sensor functionality [36]. This progress is closely linked to integrating nanomaterials and developing biomimetic materials, opening a world of possibilities and underscoring the significant role of electrochemical sensors in advancing scientific research and applications [37]. By using nanomaterials in the fabrication of the biosensor, it will enhance the sensitivity and performance of sensors. Biosensors, as biomarkers for cancer detection, are a primary example of biosensors in medical diagnosis. The electrochemical sensor based on Au-rGO composite for CA15-3 tumor is a biomarker in medical diagnosis.[38] The biosensor utilizing extremozyme represents an example of biosensor technology, particularly in environmental monitoring.[39] The food industry also utilizes biosensors for quality analysis of food materials. The biosensor based on the textile organic electrochemical transistor-based sensor is an example of a food industrial applied biosensor for quality analysis.[40]

### 2.3.2. Molecularly imprinted polymer

In material science and sensor technology, the molecularly imprinted polymers (MIP) technique is an exciting tool for making binding sites in polymer matrixes by imprinting template molecules. This method has revolutionized the field of electrochemical sensors due to its ability to improve selectivity and sensitivity without the necessity of using biological materials. The ability to engineer selective binding sites for target molecules in synthetic polymers makes this technique highly selective compared to other sensor development methods. This tailoring method, which resembles the antigen-antibody relation in a biological system, is a fascinating approach that piques the curiosity of researchers and professionals in the field. It consists of three steps: selection of template molecule, polymerization, and removal of the target molecule. These matrices are obtained by the polymerization of the monomer or electrodeposition of the polymer and its crosslinking around the template molecule to form a polymeric membrane. After polymerization, the template molecule is removed from the polymer layer, creating a binding site complementary to the target analyte molecules. The obtained polymeric layer can withstand natural and chemical variations like pH, temperature, and solvents. Thereby, this complex is chemically stable and robust.[41] Figure 3 is the schematic representation fabrication of the sensor.

Traditionally, three different synthesis methods for molecularly imprinted polymers have been classified: covalent, non-covalent, and semi-covalent bonds. This classification is based on the type of bond created between the polymer and the template. Polymers form reversible bonds between monomers and template molecules in the covalent approach. These bonds break when the template molecule is removed and reform when it reacts with analyte molecules. These bonds create a highly stable template-monomer interaction, developing a homogeneous binding site in the polymer. This type of template-monomer complex is highly reactive in mild changes in condition, thus causing difficulty in designing sensors with a covalent bond approach.[41,42]

The non-covalent bond approach forms relatively weak monomer-template molecule bonds. This technique has recently been widely used for developing MIPs. This interaction takes place before polymerization. The method used in this procedure is relatively simple and can be effective in most polymers and templates. The major drawback of this technique is that it creates a non-selective binding site due to the excess use of monomers to make template-monomer complexes. A high amount of monomer has been used to find an equilibrium in the template-monomer complex; subsequently, it causes many non-selective binding sites in the polymer [41,43].

In a semi-covalent approach, the template binds with a functional monomer in a covalent bond, but after the template is removed, the analyte binds with a non-covalent bond [44,45]. Recent developments in MIPs occur in the highly concentrated so-called molecularly imprinted solid-phase extraction, which is the most advanced and emerging application of MIPs [46–49].

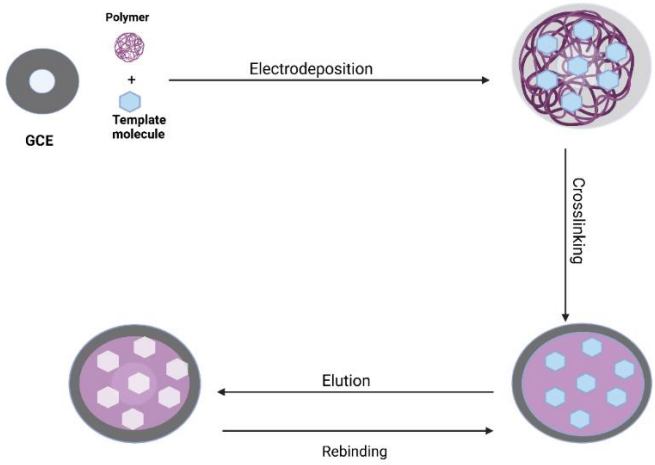


*Figure 3 Fabrication of MIP sensor*

## 2.4. Application of nanomaterial in the electrochemical sensor

An electrochemical sensor converts chemical information into an electrical signal during chemical reactions. These sensors detect various analytes, including gases, ions, and biomolecules.[50] The incorporation of nanomaterials into electrochemical sensors has led to new opportunities in terms of functionality and design.[51] Nanomaterials, with their unique properties such as large surface area, high electrical conductivity, and tunable surface chemistry, have significantly improved the performance of these sensors.[52]

The most common nanomaterials used in the electrochemical sensor are carbon-based nanomaterials, metallic nanomaterials, metal oxide nanomaterials, polymeric nanomaterials, and quantum dots.[53] Carbon-based nanomaterials include carbon nanotubes (CNTs), graphene, graphene oxide, and carbon dots.[54,55] The carbon nanotube-enhanced MIP sensor for detecting lindane is a prime example of a carbon-based nanomaterial sensor.[56] Silver and gold are widely used nanoparticles in sensor development.[57–59] The nano gold-doped MIP sensor for cortisol detection indicates the influence of the metallic nanoparticles in the development of electrochemical sensors, especially MIP sensors.[60] ZnO, $TiO_2$, and $SnO_2$ are commonly used metal oxide nanoparticles.[61] The $TiO_2$-modified MIP sensor for detecting engine oil degradation is an example of the influence of metal oxides in MIP sensor development.[62]

The primary function of nanomaterials in electrochemical sensors is to enhance their sensitivity and detection limits. It is achieved by increasing the surface area of the sensor electrode. The second main advantage of incorporating nanoparticles is the capability to improve response time. An increase in the sensor's response time occurs by reducing the detection time of the analyte by high electron transfer and catalytic properties of these nanomaterials.[63] In this project, metallic nanoparticles will be considered sensor modifiers due to their excellent properties as electron mediators.

The main challenges to integrating nanoparticles in commercial sensors are scalability, stability, and the high cost of production. Significant production of nanoparticles with consistent quality

causes scaling issues in nanomaterials. However, it has been taken into account that Metallic nanoparticle stability suffers from aggregation or degradation over time, affecting the sensor's performance.[64]

### 2.4.1. Gold Nanoparticles (AuNPs)

Gold nanoparticles are among the most studied in scientific research, classified according to their physical and biological potential and applications. Historical evidence shows that AuNPs were used from the fourth century itself [65]. Michael Faraday conducted the first major notable studies on gold nanoparticles in the 19th century. In his studies, he analyzed the optical properties of colloidal gold particles [66]. In 1951, the modern method of synthesizing gold nanoparticles was introduced, called the Turkevich method [67]. This method involves the reduction of gold salt with citrate ions to form differently shaped and sized AuNPs. The application of AuNPs has mainly lied in the biomedical field because of their biocompatibility and high interaction with biomolecules [68]. It also has a significant role in the development of nanoparticle-embedded electrochemical sensors, such as glucose sensors for the monitoring of diabetes [69], DNA sensors for finding specific genetic sequences [70], and sensors for the detection of cancer cells [71]. Another application of AuNPs in chemical analytics is environmental monitoring and food safety [72]. In environmental monitoring, nanoparticles sense heavy metals in natural resources [73].

AuNPs' central relevance in sensor development is their high surface-to-volume ratio, which can improve sensor sensitivity.[74] Another property is electrical conductivity, which is crucial in analyzing the target molecule; as a result, it affects the detection of the slightest change in the sensor. The catalytic activity of gold particles also affects the sensor sensitivity.[75]

### 2.4.2. Silver Nanoparticles (AgNPs)

Silver nanoparticles have been used since ancient times, with evidence dating back to the 4th century AD. The Lycurgus Cup, a Roman artifact, is an unintentional application of the early use of silver nanoparticles.[65] Michael Faraday's description of the colloidal properties of gold nanoparticles in 1857 laid the foundation for the modern application of metallic nanoparticles.[66] Silver Nanoparticles (AgNPs) are extensively used in various fields, including the food industry, the medical sector, and various other industries. Especially they exhibit unique physical and

chemical properties. These include optical [76], electrical conductivity [77], and biological properties [78]. Biological properties like antibacterial activity are used extensively by different industries like food, pharmaceutical, and medical sectors.[79]

Properties like large surface area and electrical conductivity show great promise in detecting trace molecules and allowing the incorporation of AgNPs into sensor development. Noble metals have plasmonic properties, enhancing the sensor's sensitive performance.[80]

## 2.6. Biopolymer

Biopolymers are naturally obtained polymers derived from living organisms that are eco-friendly and biodegradable. They can be classified into proteins, enzymes, polysaccharides, nucleic acids, etc. Another critical factor of biopolymers is biocompatibility, which increases the functionality of polymers in biological systems. In material science and biotechnology, biopolymers have increased the technological application of polymers in biological systems. Most biopolymers, especially proteins and nuclease acid, have high specificity and selectivity, enhancing the sensor's sensitivity.[81,82]

Biopolymers are highly influential in electrochemical sensors because they exhibit properties like immobilization and biofunctional coatings. The immobilization property of biopolymer created a new array of MIP sensors with a physical adsorption principle. Adding conductive materials like graphene and nanoparticles to biopolymer creates conductive biopolymer composites, increasing the sensor's response time sensitivity. The main applications of biosensors are in medical applications and food safety.[83] Medical applications mainly consisted of diagnosis purposes like glucose monitoring. In food safety, detecting pathogens from contaminated food is widely used. The influence of biosensors in environmental tracking has also been notable.[84]

Even though the use of biopolymers is a promising tool in biosensor manufacturing, some challenges remain, such as stability, shelf life, and reproducibility. Biopolymer's sensitivity to environmental conditions affects the sensor's strength and longevity, and its natural variability affects its reproducibility.[85]

### 2.6.1. Chitosan

Chitosan is a unique amino polysaccharide and biopolymer made by hydrolyzing chitin, another common natural polymer. Chitosan has various applications in different disciplines, such as biochemistry and biotechnology. Arthropods' exoskeletons and some plant cell walls are composed of chitin. It contains repeating units of 2-acetamido-2-deoxy-β-D-glucopyranose. It consists of succeeding iterations of 2-amino-2-deoxy-b-D-glucopyranose with lesser involvement of β (1,4) bonded 2-acetamido-2-deoxy-b-D-glucopyranose molecules forming a linear unbranched structure.[86] Figure 4 demonstrates the deacetylation of chitin to chitosan.

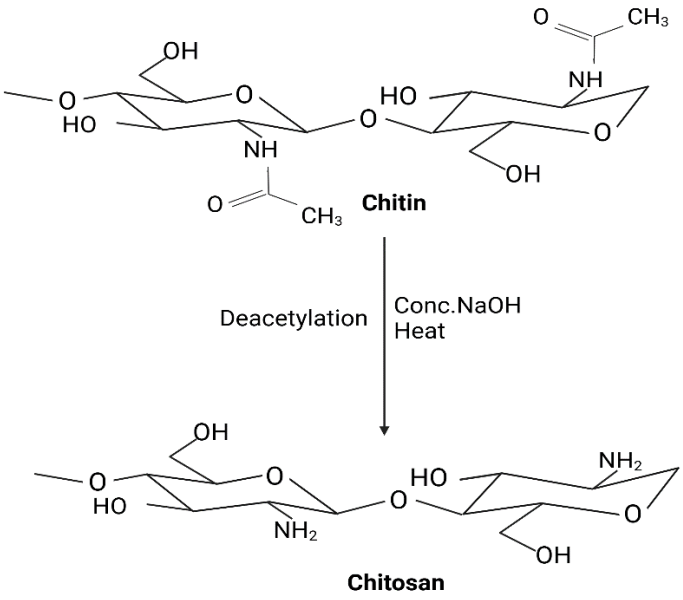


*Figure 4 Deacetylation of chitin*

The molecular weight (MW) defines the primary characterization of Chitosan. This characteristic depends fundamentally on the origin of the chitin (animal or vegetable). The degree of deacetylation (DDA) is another factor in chitosan, which is an indication of the replacement of the acetyl group with the amino group. The DDA of commercial Chitosan ranges from 70% to 90%; this parameter depends on the conditions under which Chitosan was obtained.[87] The higher the degree of DDA in Chitosan, the more crystalline the biopolymer is due to decreased acetyl groups in its chemical structure [190]. Chitosan has properties that make it an exciting biopolymer in the food industry, biomedicine, biotechnology, and the development of sensors.[88]

To obtain a crosslinked chitosan polymer layer, glutaraldehyde could be introduced as a reticulation agent. This interaction occurs when an ethylenic double bond between chitosan and glutaraldehyde forms. This bond created by the free chitosan pendant amine group reacts with the aldehydic group of glutaraldehyde, creating a stable imine bond due to the resonance phenomenon, which affects the adjacent double ethylenic bond.[89] Figure 5 represents the crosslinking of chitosan with glutaraldehyde.

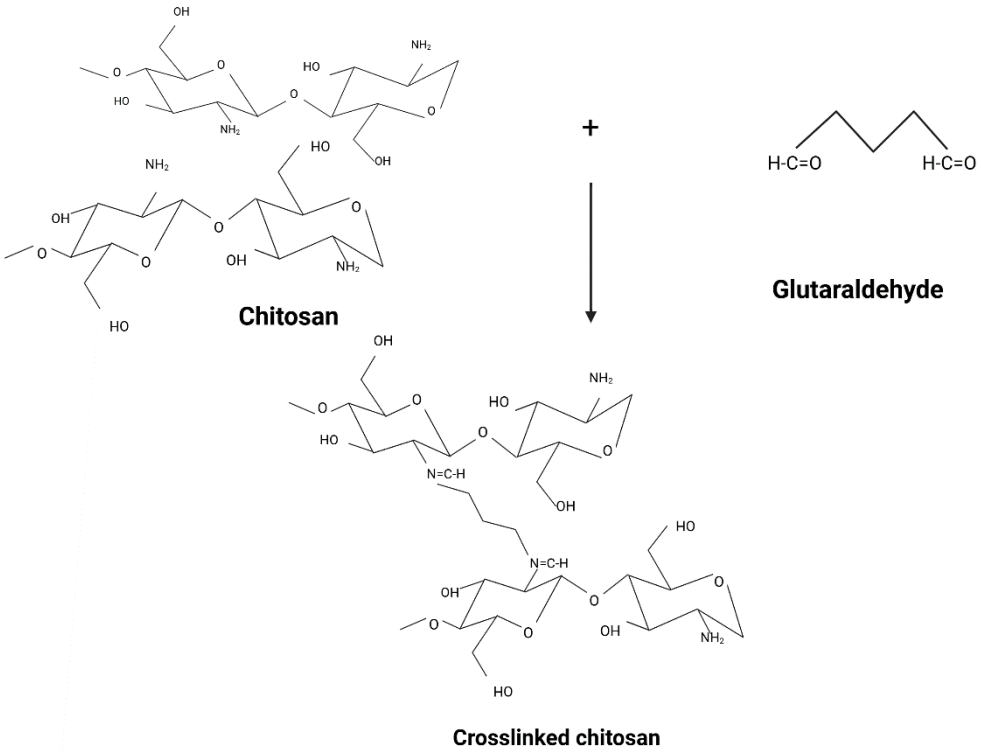


*Figure 5 Crosslinking of chitosan.*

## 2.7. Glucose and glucose sensors

Glucose is the most crucial monosaccharide in nature and is a critical factor in cellular activity. It's the main carbohydrate in food, which cells utilize as an energy source in cellular respiration [90]. Its molecular formula is $C_6H_{12}O_6$ with an aldehyde group. Because of this aldehyde group's existence, glucose is considered a strong reducing sugar. Unregulated consumption of glucose leads to various medical conditions, and diabetes is commonly called this medical condition. In the world, diabetes is classified as the fastest-growing disease and is also one of the

noncommunicable diseases out there. Due to this, developing glucose sensors is essential in medical diagnosis and the food industry [91]. Glucose has two crystalline structures: α-glucose and β-glucose, and Figure 6 represents this stereoisomer of glucose. An equilibrium obtains glucose solution; commonly, most of them are β-glucose, and a small amount of α-glucose is also present. This difference occurs because of the mutarotation phenomenon, in which the dissolved α-glucose molecule turns into a β-glucose form [90]. The normalized form of sugar is an odorless solid in a white crystalline powder. It's a highly water-soluble crystal with 91 g per 100 ml of water. It has a molecular weight of 180.16 g/M and a melting temperature of 146°C.

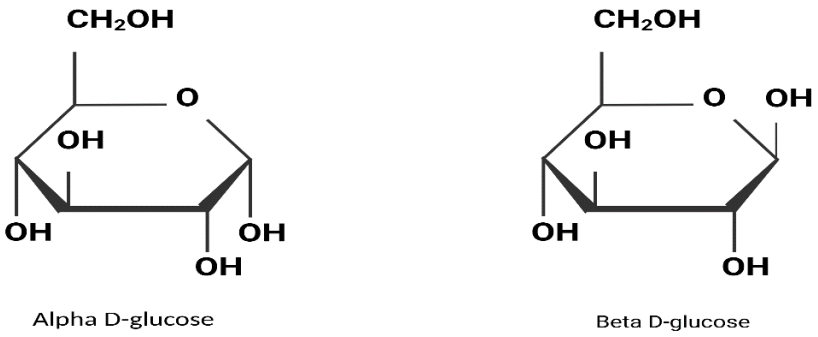


*Figure 6 Stereoisomers of glucose.*

The most commercially available glucose sensor works under the principle of enzymatic biosensor proposed by Clark 62 years back.[92–94] Enzymatic sensors' main drawback is their low functional period, which limits their continuous application. Poor stability is also a limiting factor.[95] This type of sensor is highly vulnerable to environmental changes. To overcome these disadvantages, non-enzymatic sensors were developed. With the aid of nanomaterials, a new generation of glucose sensors emerged.[96]

Recently, more and more MIP-based sensors have been developed for the detection of glucose. This sensor has been created by coating electrodes with MIP layers, which offer recognized sites for glucose molecules with higher selectivity and stability. The laser-pyrolyzed paper analytical electrodes (LPPEs)[97] with the molecularly imprinted layer of glucose by 3-amino-4-hydroxybenzoic acid monomer are one of the most recent developments in MIP sensors for glucose. Other recent advances in MIP sensors for glucose detection, including the CuCo

bimetal-coated with a glucose-imprinted polymer (GIP) and the chitosan-based MIP sensor with nickel oxide electrodes some other recent developments in MIP sensors for glucose detection.[98,99]

## 2.8. Lactose and lactose sensor.

Mammary glands in mammals produce milk, which naturally contains 2 to 8 % lactose, making it an animal-derived sugar. The International Union of Pure and Applied Chemistry (IUPAC) has named lactose β-D-galactopyranosyl — (1→4)-D-glucopyranose, a disaccharide composed of monosaccharide units of glucose and galactose. This composition is due to the β (1,4) glycosidic enzyme in milk, which helps humans break down lactose into its monosaccharide units 80,81. This breakdown of lactose is shown in Figure 7.

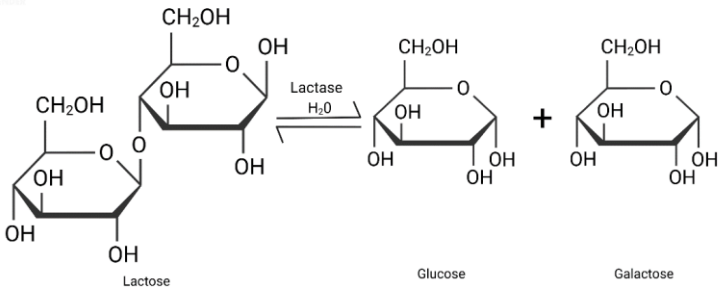


*Figure 7 Breakdown of lactose*

Mutarotation at room temperature results in 60% β-lactose and 40% α-lactose in lactose. Figure 8 shows the reaction that takes place in the mutation process. The presence of a high reactive carbonyl group makes lactose a reducing sugar.[100] Lactose is usually found in the atmosphere as a white crystalline powder with a melting point of 252ºC and a high solubility of 50 g per 100 g of water. Its molecular weight is 342.3 g/mol.

The

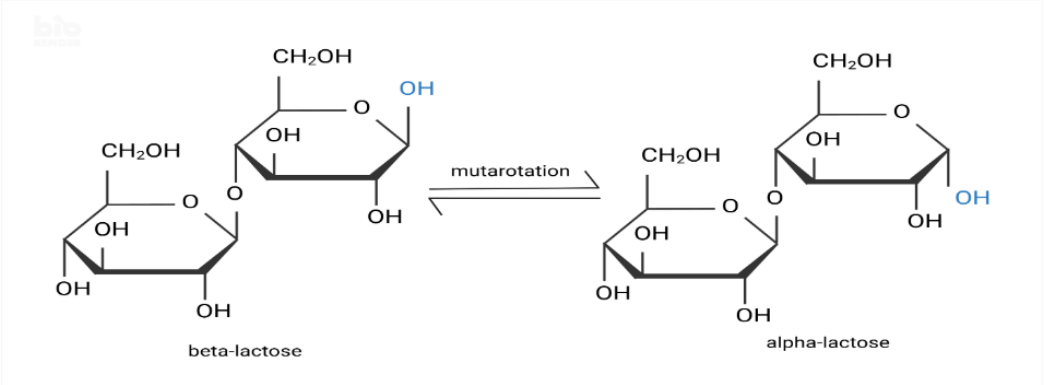


*Figure 8 Mutarotation of lactose*

classification of the diary product is crucial in modern society because of the increase in lactose intolerance pupils. Therefore, it is essential to monitor lactose in dairy products even though many conventional methods exist to estimate the lactose content. [101,102] This method's limitation is that it is not feasible for continuous monitoring. Thus, electrochemical sensors are a valuable option compared to traditional analytical practices. [103,104] This leads to the creation of the electrochemical sensor with nanoparticles and the development of a non-enzymatic sensor.

Among non-enzymatic sensors, the molecularly imprinted polymer is a novel technique used to develop lactose sensors. The graphite paper electrode (PE) sensor with polypyrrole (Ppy) is a recently developed MIP sensor for lactose detection.[105] This sensor exhibited excellent stability, reproducibility, and repeatability. Its exceptional performance in the food sample led to its industrial application.

# CHAPTER 3

# Reagents, Materials & Equipment

# 3. Reagents, Materials & Equipment

## 3.1. Reagents

The reagents used in the experiment are mentioned below:

- Acetone ($C_3H_60$, purity of 99%, Quality Chemicals, CAS Number: 67-64-1).
- Alpha-D (+)-glucose ($C_6H_{12}O_6$, purity >99%, Sigma-Aldrich, CAS number 492-62-6).
- Chitosan ($C_{56}H_{12}O_6$, purity>99%, Sigma-Aldrich, CAS number: 9012-76-4).
- Glacial acetic acid ($CH_3COOH$, purity of 99.7%, Panreac, CAS Number: 613-90-4).
- Glutaraldehyde ($C_5H_8O_2$, 50% aqueous solution, Alfa Aesar, CAS Number:111-30-8).
- Gold chloride trihydrate ($HauCl_4$ $3H_2O$, CAS number: 16961-25-4).
- Lactose ($C_{12}H_{22}O_{11}$, Sigma-Aldrich, CAS number: 63-42-3)
- Milli-Q deionized water (resistivity 18.2 MΩ cm)
- Potassium chloride (KCl, Sigma-Aldrich, CAS number: 7447-40-7)
- Silver nitrate ($AgNO_3$, Sigma-Aldrich, CAS Number: 7761-88-8)
- Sodium borohydride ($NaBH_4$, Sigma-Aldrich, CAS number 16940-66-2)
- Sodium dihydrogen phosphate ($Na_2H_2PO_4$, minimum purity of 99%, Sigma Aldrich, CAS number 7558-79-4).
- Sodium hydrogen phosphate ($Na_2HPO_4$, minimum purity 99%, Sigma-Aldrich, CAS number: 7558-80-7).

## 3.2 Materials

- 50 mL electrochemical cell.
- Glassy carbon electrode (working electrode) (GC)
- Hot plate magnetic stirrer
- Platinum plate (2 cm x 1 cm) (auxiliary electrode)
- Reference electrode (Ag/AgCl)
- Silicon Carbide grinding papers

## 3.1. Equipment

- 34972A LXI Data Acquisition/Data Logger Switch Unit (Keysight Technologies, California, USA)
- MicropH 2000 pH meter (Crison, Barcelona, Spain)
- PGSTAT128 Potentiostat/Galvanostat (AutolabMetrohm, Utrecht, The Netherlands)
- Ultraviolet-visible spectrophotometer model UV-2600 (Shimadzu Corporation, Japon).

# CHAPTER 4

# Methodology

# 4. Methodology

## 4.1. Development of Chitosan nanoparticle MIP Sensor

The electrochemical sensors were developed using the basics of the molecularly imprinted polymer technique. The biopolymer chitosan was used to be imprinted, and two different sugars, glucose or lactose, were used as template molecules. Moreover, the polymer matrix can be modified with metallic nanoparticles (gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs)), which have been evaluated. The experimental analysis was conducted using potentiometry or voltammetry techniques. In each study, the sensors were compared to polymeric membranes developed in the presence of a template molecule (MIP) and the absence of a template molecule (NIP).

### 4.1.1. Preparation of AgNPs

For the synthesis of silver nanoparticles, the procedure developed by S.D Soloman et al. I, is adopted.[106] This technique uses sodium borohydride ($NaBH_4$) as a reducing agent and silver nitrate ($AgNO_3$) as the silver ion source.

- To prepare AgNPs, 30 mL of 2 mM freshly prepared sodium borohydride is added to an Erlenmeyer flask. Then, a magnetic stir bar is added, and the flask is placed in an ice bath over the stir plate. The ice bath reduces the decomposition of silver nanoparticles during the experiment.
- The second step is the addition of the silver nitrate ($AgNO_3$). For that, 10 mL of 1 mM $AgNO_3$ is added drop-by-drop using a glass pipette to the string Erlenmeyer flask, and this addition takes around 3 minutes.

After adding the silver nitrate to the Erlenmeyer flask, the colour changed from a noncolored solution to pale yellow in colour. After colour formation stirring is stopped and the colloidal solution is stored in cold temperature without exposure to sunlight.

### 4.1.2. Preparation of AuNPs

For the synthesis of gold nanoparticles, Turkevitch et al.'s [67] method is commonly used, or a modified version of Turkevitch was used.

- In an Erlenmeyers flask, 25 mL of 0.25 mM $HauCl_4$ is added, and the solution is stirred by adding a magnetic stir bar and setting a 150ºC thermal resistance.
- After the solution boils, 180 µL of a 17mM trisodium citrate solution is added to the Erlenmeyres flask drop by drop. The stirring continues for around 20 minutes. After the solution turns colorless to red, remove the heat from it.

After obtaining gold nanoparticles, they are stored at an 8 ºC temperature in a refrigerator

### 4.1.3. Sensor development

In the fabrication of MIP sensors, the electrodeposition of the polymeric membrane on the electrode is the first step. For that, it is necessary to conditionate the commercial GCE

- **Cleaning of the glassy carbon electrode (GCE)**

Before starting the electrodeposition process, the surface of the electrode must be thoroughly cleaned—this is a critical step for ensuring the reproducibility and reliability of the sensor readings. Chemical and physical cleaning methods are used to provide this procedure. The chemical method involves an ultrasound of the GCE in the presence of ethanol or Milli Q deionized water for 10 minutes. Before the implementation of this chemical treatment, the physical method was effectively utilized. The physical method comprises electrode modifiers like diamond polish and polishing alumina for the surface electrode polishing pad. The polishing starts with P1200 grit sandpaper. To maintain consistency in polishing, an infinity symbol-like pattern is followed. After successive polishing, the electrode surface is rinsed with Milli Q water. Subsequently, diamond polishing is executed in the nylon diamond polishing pads, commencing with a coarse diamond polish and progressing to a fine diamond polish. The final step in the polishing process involves the application of alumina slurry on alumina polishing pads to polish the electrode. Each polishing takes around 1 minute.

- **Preparation of polymeric solution for electrodeposition**

The main component of the electrolyte for electrodeposition preparation is chitosan polymer. For that, chitosan is diluted in the acetic acid phosphate buffer solution. 1.5 mg of chitosan is diluted in 1 ml of 30 % acetic acid. This acetic acid is prepared with 10.3 mM phosphate buffer of pH 7: 30 ml of acetic acid mixed with 70 ml of phosphate buffer. This phosphate buffer is prepared with 3.8 mM $Na_2HPO_4$, 6.5 mM $NaH_2PO_4$, and 0.1 M NaCl salt. The obtained chitosan solution will have a pH range of 1 to 2. Based on the previous investigation in our group, researchers found that the pH 4 chitosan solution was better for the electrodeposition. Therefore, the pH of the chitosan solution is changed using 2 M NaOH.

To prepare the MIP sensors, the template molecule must be added to the chitosan solution. Depending on the sensor, different types of electrolyte solutions are used.

- A concentration of 0.1 M glucose is added (1.8 g of glucose in 100 ml) into the chitosan solution to obtain the chitosan-modified MIP sensor for glucose detection.
- The chitosan-modified MIP sensor for lactose detection is prepared in the concentration of 0.1 M lactose (3.4 g of lactose in 100 ml of chitosan solution) in chitosan solution.

The non-imprinted polymer sensors are the sensors that were developed without template molecules. For nanoparticle-modified NIP sensors, the prepared stock solution of chitosan is mixed with metallic nanoparticleS solutions in a ratio of 1:2(150 mL solution consists of 50 mL nanoparticles and 100 mL chitosan).

For the nanoparticle-modified MIP sensor, after obtaining the matrix of nanoparticles with chitosan, the addition of a template molecule with the desired concentration is prepared.

- The AuNPs-modified MIP sensor for glucose is developed by making a matrix of chitosan, AuNPs, and 0.1 M glucose. For that, 1.8 g of glucose is mixed with a stock solution of 100 ml OF .2 mM AuNPs mixed with chitosan in a 1:2 ratio to make an AuNPs-modified MIP electrolyte.
- To make AuNPs-modified MIP electrolytes of 0.1M lactose, 3.4 g of lactose was mixed with a stock solution containing 0.2 mM AuNPs and chitosan.

The same conditions are used for making 0.25 mM AgNPs-modified MIP electrolytes. The only change is in preparing the stock solution, which is a change in the nanoparticle. Therefore, 50 mL AgNPs colloidal solution nanoparticles are added to 100 mL chitosan (ratio 1:2).

- **Electrodeposition**

The polymeric layer is deposited on the electrode's surface using cyclic voltammetry to develop an MIP or an NIP sensor. In this study, different types of polymeric matrixes are deposited on the surface of the glassy carbon electrode using potentiostat/galvanostat equipment.

According to the sensor, a different electrolyte is present in the electrolytic cell for the electrodeposition of the polymer. For cyclic voltammetry, a three-electrode is presented in the electrolytic cell. Glassy carbon is the working electrode, and the reference electrode is an Ag/AgCl reference electrode in KCl. A platinum sheet is used as a counter-electrode. The process involves pouring a suitable electrolyte of 5 mL into the electrolytic cell. An electrolyte with a template is used for MIP, whereas for NIP, an electrolyte without a template is used.

The parameters for the cyclic voltammetry are:

- Number of cycles: 10
- Upper vertex potential: 0.5 V
- Lower vertex potential: -1.5 V
- Starting and stopping potential: 0 V
- Scan rate: 0.1 V/s

- **Crosslinking**

After the electrodeposition, the deposited polymeric film must be crosslinked with the polymeric chain of chitosan. Glutaraldehyde is used as the crosslinker in the chitosan polymer. Therefore, after electrodeposition, the electrode is exposed to a 50 % aqueous solution of glutaraldehyde vapour for 20 minutes.

- **Elution**

Elution is a process used to remove the template, creating cavities/holes in the polymeric membrane specific to the target. In this process, the template molecule is dissolved in the eluent. In this study, 0.1M NaOH . For the elution, the electrode is submerged in the eluent for 10 minutes.

### 4.1.4. Sensor detection

#### 4.1.4.1 Potentiometry response

Potentiometry has been one of the analytical tools used to analyze the performance of the developed sensors. After electrodeposition, crosslinking, and elution, the sensor is analyzed in a standard solution. In the elution step, the template molecule is removed from the polymer film, generating holes in the membrane. During in the analysis, the analyte molecule will fill this void producing an electrochemical responseThis electrochemical change can be analyzed by potentiometry. Different types of analyte concentrations ($10^{-2}$ to $10^{-5}$ M) are analyzed for quantitive analysis.

Two electrodes are present in potentiometry for analysis in the electrolytic cell; the working electrode is GCE with a polymer layer, and the reference electrode is Ag/AgCl in the KCl electrode. The scanning time of potentiometry is 5 minutes. The sensor's response is read from lower analyte concentrations to higher concentrations.

#### 4.1.4.2 Cyclic voltammetry response

As in the previous paragraph, the NIP and MIP conduct a qualitative analysis, and the different analyte concentrations ($10^{-2}$ to $10^{-5}$ M) perform quantitive analysis. In that case, cyclic voltammetry is used to determine the redox activity that is placed on the electrode surface in the presence of the analyte.

Applied condition for cyclic voltammetry:

- Number of cycles: 5
- Upper vertex potential: 1.5 V
- Lower vertex potential: -1.5 V

- Starting and stopping potential: 0 V
- Scan rate: 0.1 V/s

## Chapter 4

# RESULT AND CONCLUSION

# 5. Result and discussion

The results of these experiments are mainly classified based on chitosan-modified sensors developed in the presence or absence of metallic nanoparticles. Further, the response of these electrochemical sensors has been registered differently based on electrochemical methods. That means voltammetry and potentiometry methods are used to evaluate the sensor performance. In each process, two types of sensors have been developed: MIP, and NIP. These sensors have been designed to assess the capability of MIP to detect sugar, such as glucose and lactose.

The first part of the experiment mainly concentrated on optimizing the performance sensor. Therefore, different parameters and synthesizing techniques are used to optimize the sensor. First, it has been optimizing the electrodeposition of the polymeric membrane onto the electrode.

## 5.1. Electrodeposition

In this procedure, the polymer is electrodeposited on the surface electrode using Cyclic Voltammetry. For the development of the MIP sensor, chitosan was also deposited in the presence of a template molecule (lactose or glucose). In the case of nanoparticle-based MIP, the electrodeposition occurs in the presence of nanoparticles. Also, in the case of the NIP sensor, the electrodeposition occurs in the presence of nanoparticles but, in all cases, without a template molecule.

### 5.1.1. Electrodeposition of Chitosan-modified MIP sensor to detect glucose

The electrodeposition of the Chitosan-modified MIP sensor is carried out in the presence of a template molecule, glucose. The electrodeposition had an applied voltage of 0.5 to 1.5 V. After physically and chemically cleaning of electrode's surface, the polymer is electrodeposited using cyclic voltammetry. Figure 9 depicts the voltammograms obtained for NIP and for MIP GCE modified sensors. The intensity current after each cycle. This implies a properly executed electrodeposition.

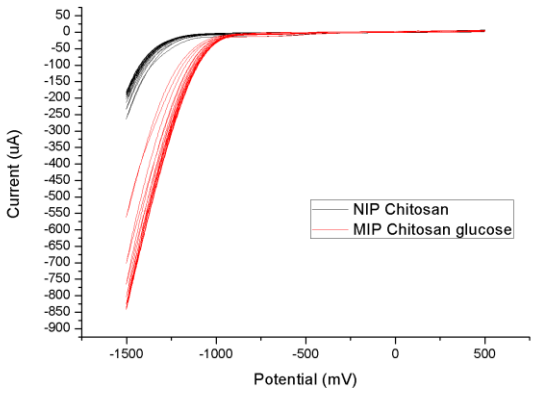


*Figure 9 Electrodeposition Of chitosan-modified NIP and MIP sensor. (Overlayed)*

The overlayed voltammogram in Figure 9 of NIP and MIP indicates that the intensity of the current changes according to the MIP and NIP sensors.

### 5.1.2. Electrodeposition of NPs-modified MIP sensor to detect glucose.

In the nanoparticle-modified MIP sensor and NIP sensor, the applied potential remains constant at -1.5 to 0.5 V. Even if the condition remains constant, the matrix solution used in the electrodeposition includes metallic nanoparticles, as it has been described in the methodology section.

As Figure 9 shows, the voltammogram obtained from the electrodeposition of the NPs modified NIP and MIP sensor presents a significant difference compared to the non-modified MIP and NIP sensor. The main difference is that for the AgNPs-modified sensor, the NIP sensor electrodeposition voltammogram presents a higher intensity than the MIP sensor. In the analysis of nonmodified sensors, MIP sensors always have a higher intensity than NIP sensors. This trend is repeated in further studies. A possible explanation for such a response could be the influence of NPs on the NIP sensors to increase their conductivity. In contrast, the MIP sensor is developed with template molecules and nanoparticles. Therefore, the MIP sensor has fewer nanoparticles than the NIP sensor.

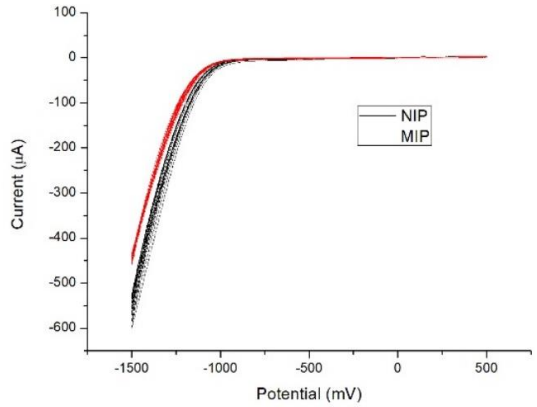


*Figure 10 Electrodeposition of AgNPs-modified MIP and NIP sensor (overlayed)*

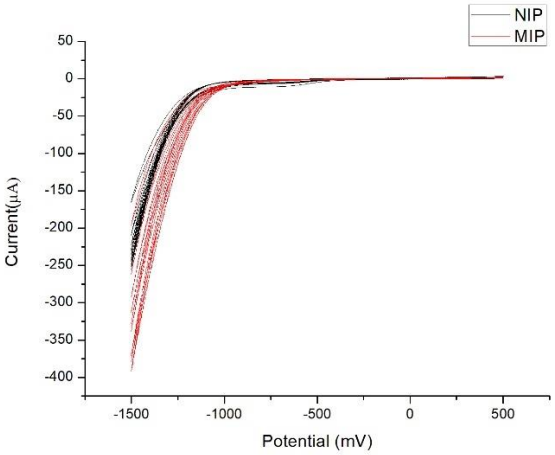


*Figure 11 Electrodeposition of the AuNPs-modified NIP and MIP sensor.*

As Figure 11 shows, AuNPs' modified NIP and MIP sensor electrodeposition varies from that of AgNPs' modified sensors. In AgNPs, NIP has a higher current intensity than MIP. However, in AuNPs, MIP has a higher current intensity than NIP.

The comparative analysis of MIP and NIP based on chitosan, chitosan-AuNPs, or Chitosan-AgNPs gives a general overview of the presence of nanoparticles and template molecules in the polymer matrix. It is possible that the applied conditions of cyclic voltammetry will remain constant. Therefore, changes in the polymeric matrixes are evident in the voltammogram of the individual sensors. These changes can happen through variations in the electrode surface. This point of difference can be avoided by selecting an electrode in the fabrication sensors. Also, the influence of a change in pH can change the polymerization.

### 5.1.3. Electrodeposition of Chitosan-modified MIP sensor to detect lactose.

Based on previous investigations with glucose templates, working with AgNPs nanoparticle-modified and nonmodified MIP sensors is ideal. The voltammogram in Figure 12 obtained from the lactose sensor resembles the voltammogram of the glucose sensor.

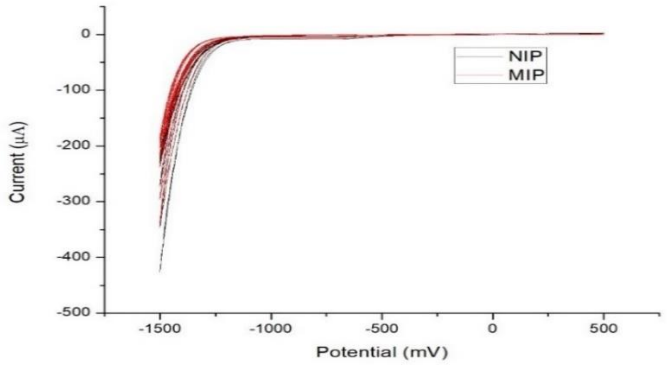


*Figure 12 Electrodeposition of Chitosan-modified MIP and NIP sensor to detect lactose (overlayed)*

The lactose non-modified sensors' electrodeposition voltammograms are entirely different from those obtained in the other electrodeposition. The overlayed MIP and NIP voltammogram reveals that MIP and NIP are closely alike, with NIP having a higher intensity than MIP. Even with this, no variable reduction or oxidation peak is observed.

### 5.1.4. Electrodeposition of AgNPs-modified MIP sensor to detect lactose.

The lactose AgNPs modified sensor electrodeposition voltammogram in Figure 13 shows a similar trend to that for glucose detection. In this case, the NIP sensor also has a higher current intensity than the MIP. Based on this finding, this difference in the voltammogram occurs due to the AgNPs in the matrix. Another significant difference between modified and non-modified sensor electrodeposition is the MIP and NIP have a change in intensity in the cathodic region. This change in intensity is typically observed in all electrodepositions in the experiment. However, this change is the least observed in the nonmodified lactose MIP and NIP sensor electrodeposition.

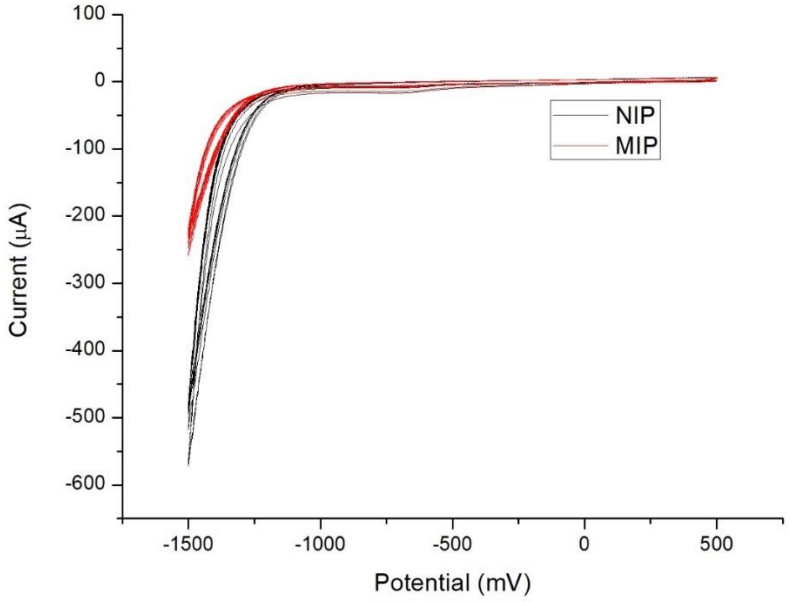


*Figure 13 Electrodeposition of the AgNPs-modified MIP and NIP sensors to detect lactose.*

By analyzing all voltammograms obtained during electrodeposition, we infer that the presence of template molecules and nanoparticles can influence polymerization. Furthermore, nanoparticles will reduce current intensity in the cathodic area. MIPs have a higher current intensity in nonmodified glucose sensors when AgNPs are introduced to the same electrolyte solution. NIPs will have more significant cathodic peaks, while MIPs will be less intense. Similar behavior is observed with glucose silver nanoparticle-modified sensors. As mentioned in the glucose sensor, NIP in the nanoparticles-modified has many more nanoparticles than the MIP sensor. The same phenomenon repeated in both lactose and glucose. In that case, the influence of nanoparticles changes the peaks in the nanoparticle-modified sensor and chitosan-modified sensor.

The electrodeposition is the primary step in optimizing the MIP sensor. After eluting the template molecules, the primary analytical studies will be carried out later using potentiometry or cyclic voltammetry. For that, potentiometry or voltammetric technique is implemented.

## 5.2. Sensing behaviour of the Chitosan MIP sensor for sugar detection

Once the sensors (MIPs and NIPs) are obtained from successful electrodeposition, the template molecule is removed from the sensor using 0.1 M NaOH for 10 minutes. After that, the sensor is analyzed using the standard glucose solution to measure the sensor's response. Cyclic voltammetry or potentiometry is used for that.

### 5.2.1. Sensing behavior of the Chitosan modified MIP for Glucose detection.

#### Potentiometric response.

After successful electrodeposition, the potentiometric response of the sensor has been analyzed to evaluate the analytical behavior of the chitosan-modified MIP sensor. Figure 14 depicts the potentiometric response of the MIP and NIP to glucose solutions ranging from $10^{-2}$ M to $10^{-5}$ M. This graph shows a clear difference in the intensity of NIP and MIP. The slope increase of the MIP response reveals that the MIP sensor responds to the concentration of glucose solution. This graph also displays the effectiveness of the MIP sensor.

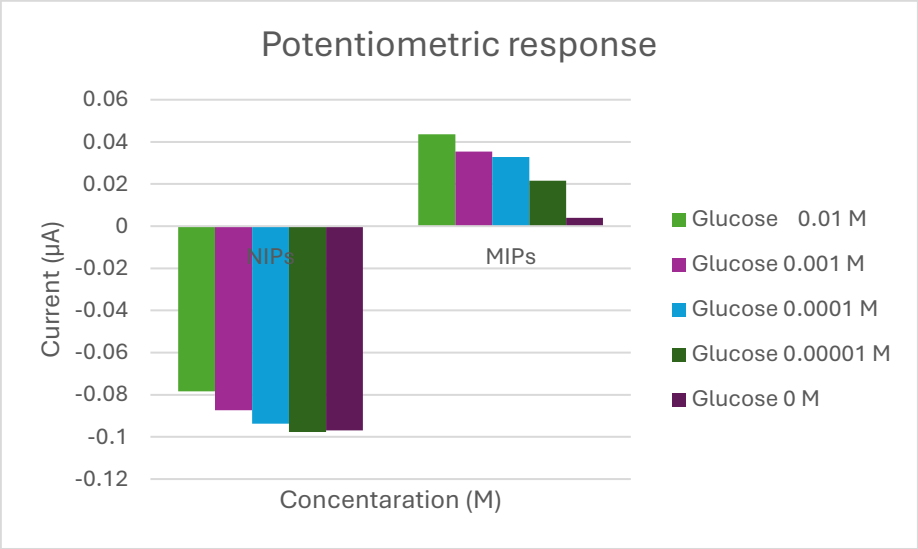


*Figure 14 Potentiometric response of chitosan-modified MIP and NIP towards glucose solution.*

It is observed that the intensity current increases with the concentration, in the case of MIP, to positive values, demonstrating the sensor's capability to detect glucose, whereas for NIP, the value is negative, and the linearity is worse.

This rise in MIP response creates the MIP sensor's calibration curve. The concentration range is from $10^{-2}$ to $10^{-5}$ M. Figure 15 depicts the calibration curve of the chitosan-modified MIP sensor. Figure 16 depicts the achieved calibration curve for the chitosan-modified NIP sensor towards glucose.

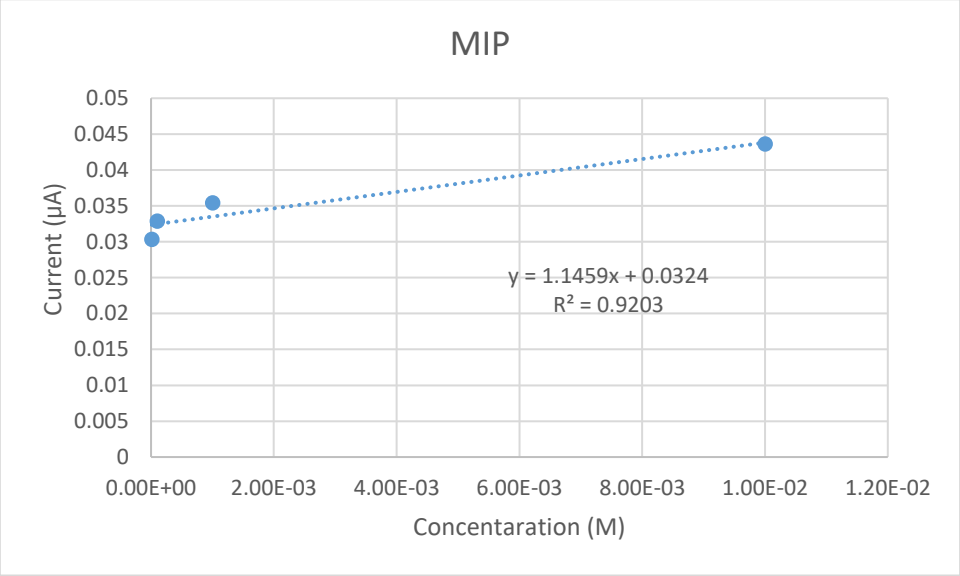


*Figure 15 calibration curve of chitosan-modified MIP sensor to detect glucose*

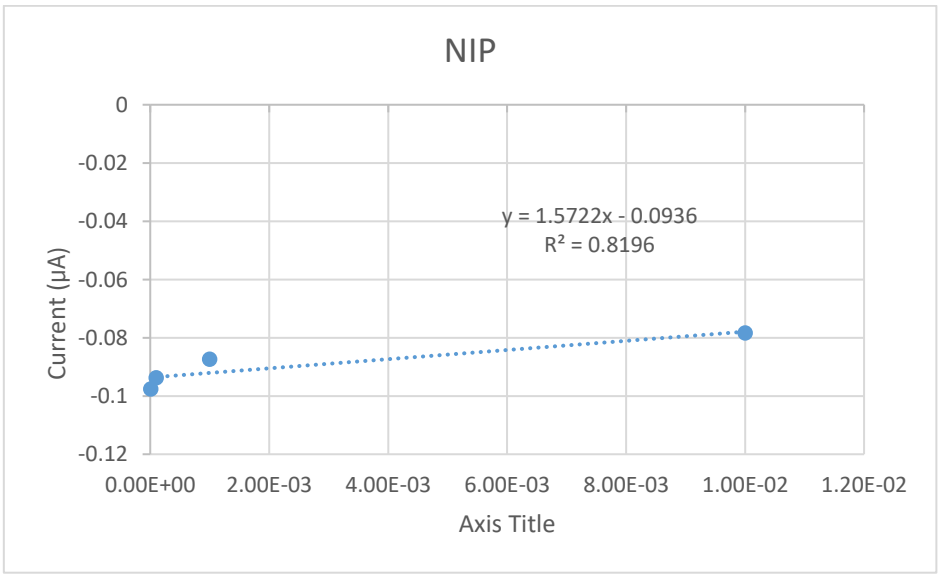


*Figure 16 calibration curve of chitosan-modified NIP sensor to detect glucose*

The obtained calibration curve is an aid in calculating the MIP sensor's detection limit (LOD).LOD is a statistical method to measure the lowest quantity or concentration of a substance reliably detected by the sensor. To calculate the limit of detection, the following formula is used:

$$\mathrm{LOD} = \frac{3 \times \sigma}{\mathrm{slope}}$$

where σ is the standard deviation obtained by calculating and measuring the blank, which the sensor responds to 0.1 M KCl; the slope is obtained from the calibration curve.

*Table 1 LOD calculation for glucose*

| Glucose | Slope | $R^2$ | Standard deviation | LOD (M) |
|---|---|---|---|---|
| **$10^{-2}$ M – $10^{-5}$ M (MIP)** | 1.1459 | 0.9203 | 8.08585E-05 | $2.09 \cdot 10^{-4}$ M |
| **$10^{-2}$ M – $10^{-5}$ M (NIP)** | 1.5722 | 0.8196 | 0.000847341 | $1.6 \cdot 10^{-3}$ M |

Table 1 shows the LOD of the chitosan-modified MIP and NIP sensor for detection. The LOD MIP sensor is $2.09 \cdot 10^{-4}$ M LOD after analyzing $10^{-2}$ to $10^{-5}$ M glucose solution, which is lower than the corresponding NIP sensor. It is observed that specific cavities improve the analytical performance of MIP sensors compared with those without cavities.

**Cyclic Voltammetry response.**

As in the potentiometry, the sensors in a standard glucose solution were analyzed using cyclic voltammetry. In voltammetry, the sensor response is obtained as a voltammogram. Figure 17 shows the voltammogram of the NIP and MIP sensors towards a glucose solution of $10^{-2}$ M concentration. Based on this figure, in $10^{-2}$ M concentration, the intensity of current obtained with the MIPs sensor is slightly higher than in the cathodic and anodic regions compared with the obtained with NIP. It indicates the presence of the holes created by the template molecule in the MIP sensor. The observed two peaks correspond to the oxidation and reduction of $H_2O_2$ during the glucose reaction over the applied range potential between -1.5 and 1.5 V.

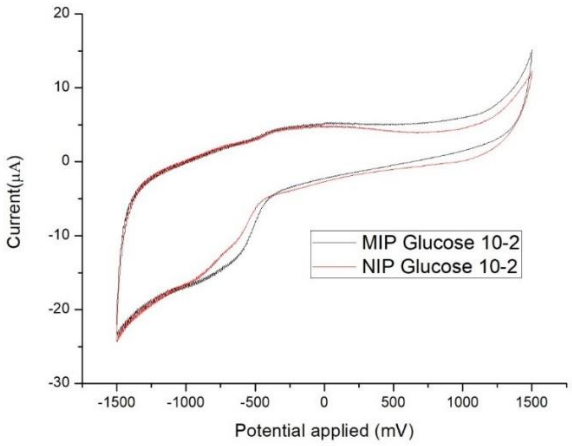


*Figure 17 Cyclic Voltammetry response of the chitosan-modified MIP and NIP towards a $10^{-2}$ glucose solution.*

The sensor response to increasing concentrations of glucose has been analyzed. The peaks in the voltammogram rise progressively with concentration. This differentiation of peaks corresponding to concentration aids in preparing the calibration curve of the MIP sensor. This study calculated the curve from the maximum reduction peak obtained at -715 mV. The analyte concentrations range from $10^{-2}$ to $10^{-5}$ M. Figure 17,18 shows the calibration curve of the MIP and NIP sensors.

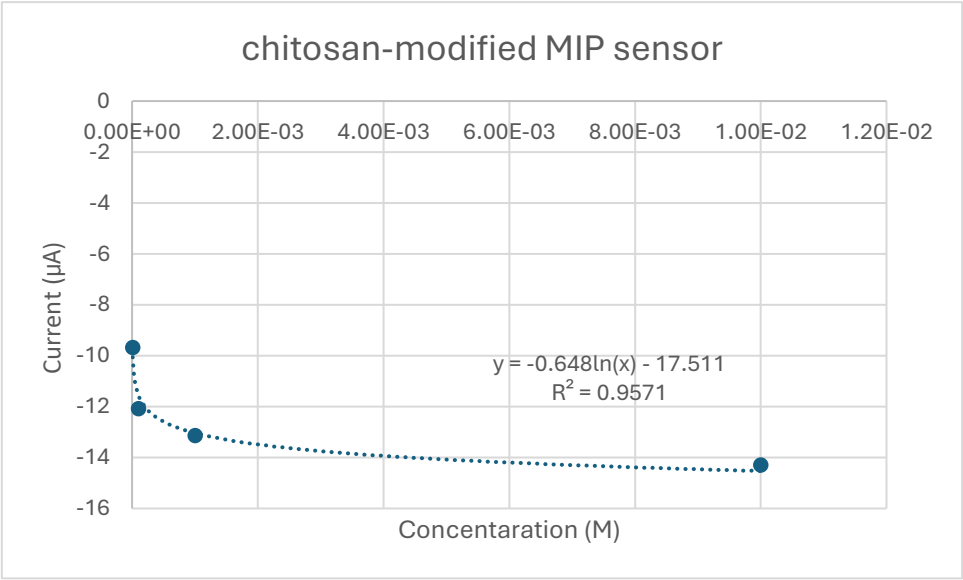


*Figure 18 calibration curve of chitosan-modified MIP sensor to detect glucose*

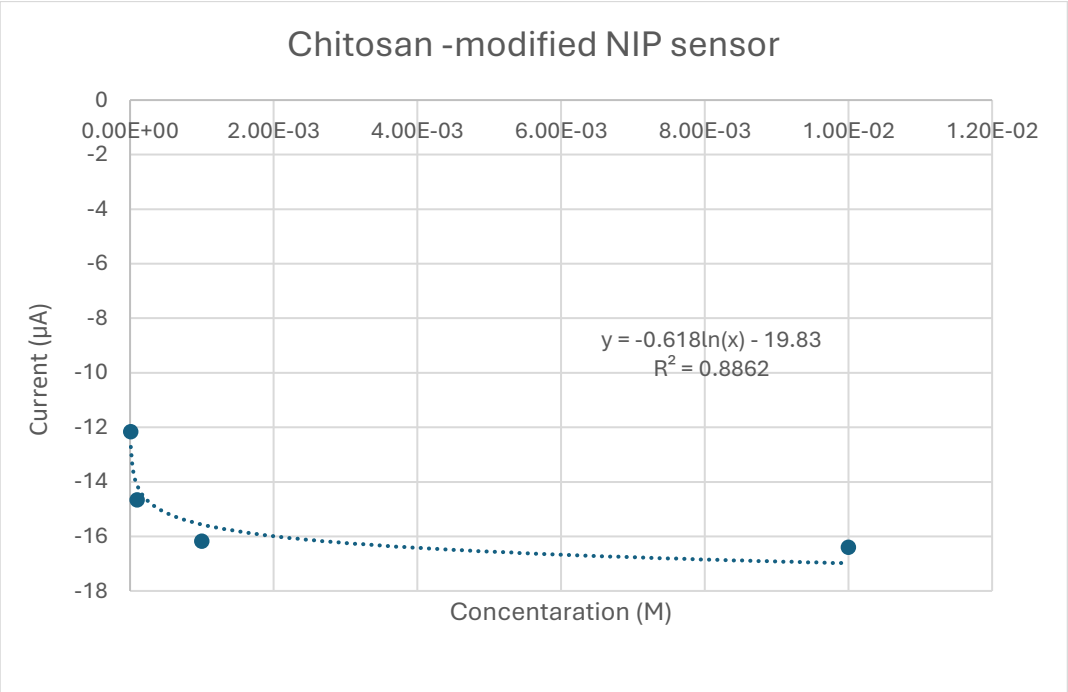


*Figure 19 calibration curve of chitosan-modified NIP sensor to detect glucose*

The obtained calibration curves are not a linear trend; for further clarification, the logarithmic trendline is used in Figures 18 and 19. Therefore, when calculating LOD from a logarithmic equation, the following formula is used:

$$\mathbf{LOD = e\ \frac{3\times \sigma\text{-}d}{slope}}$$

In the calibration curve logarithmic trendline y = a·ln(x)+b

Where:

- y is the measured signal
- x is the concentration of the analyte
- a is the slope
- b is the intercept

Using this formula, Table 2 is obtained.

*Table 2 LOD of chitosan-modified MIP sensor towards glucose solution.*

| **Glucose (Cyclic voltammetry)** | **Slope** | **$R^2$** | **Standard deviation** | **LOD (M)** |
|---|---|---|---|---|
| **MIP ($10^{-2}$ M – $10^{-5}$ M)** | -0.648 | 0.95571 | | **$6.9 \cdot 10^{-13}$ M** |
| **NIP ($10^{-2}$ M – $10^{-5}$ M)** | -0.618 | 0.8862 | 0.459513 | **$9.3 \cdot 10^{-14}$ M** |

The obtained LOD is unrealistic because the LOD concentration is much lower than the range of concentration of the sensor response. This investigation analyzed the sensors in the $10^{-2}$ to $10^{-5}$ M range. The logarithmic trendline can provide a better fit than linear by accommodating sudden changes in the concentration. Therefore, the obtained LOD is a theoretical prediction rather than a practical or experimental one. Even though the LOD states a lower value, the particle value will remain between the analyzed concentration.

### 5.2.2. Sensing behavior of the AuNPs modified MIP for Glucose detection.

After analyzing the chitosan-modified glucose sensors, the fabrication new type of sensor with nanoparticles is developed to investigate the influences of nanoparticles in the development of the sensor. For that purpose, metallic nanoparticles such as AuNPs and AgNPs were introduced, which have an electrocatalytic effect due to the reactivity of the surface usually linked to the existence of a mixed valence state. Therefore, AuNPs and AgNPs are used to improve the intensity of the signals of the newly developed sensors.

After preparing the colloidal AuNPs by reduction of tetrachloroauric acid ($HauCl_4$) using sodium citrate. This colloidal AuNPs is mixed with 0.1 M glucose chitosan solution with a ratio of **1:2 (AuNPs: CS).**

Once the successful electrodeposition AuNPs-modified MIP sensor is, this sensor is then analyzed in standard solution glucose. Figure 19 is the potentiometric response of the AuNPs-modified MIP and NIP sensor. Figure 20 was obtained by analyzing the standard solution of glucose from **$10^{-2}$ to $10^{-5}$ M.**

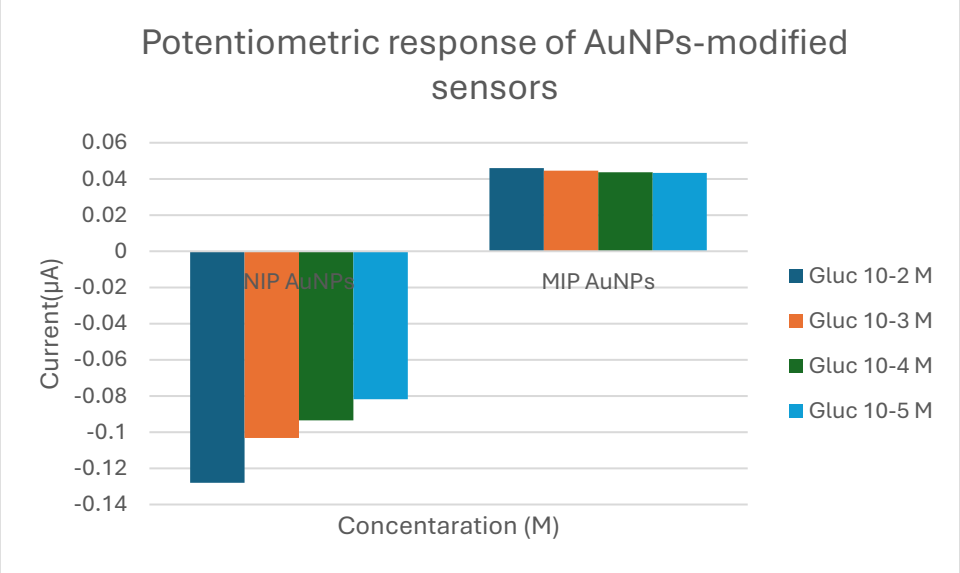


*Figure 20 Potentiometric response of AuNPs modified NIP and MIP towards glucose solution.*

A calibration curve of the MIP sensor was prepared based on this potentiometric response of the AuNP-modified sensor. Figure 22 shows the obtained calibration curve of the MIP sensor. This calibration curve was obtained by analyzing the glucose solution from 10-2 to 10-5 M. Figure 22 represents the calibration curve of the AuNPs-modified NIP sensor.

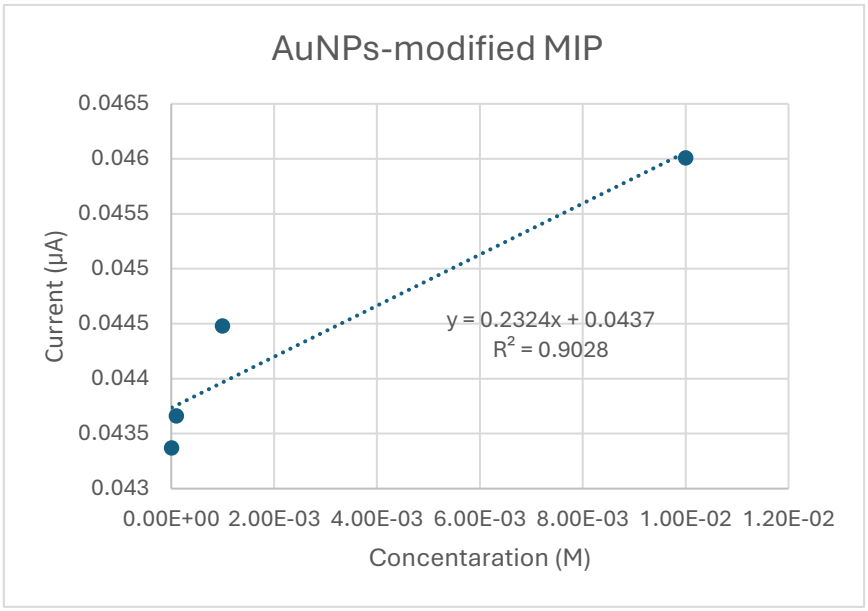


*Figure 21 Calibration curve of AuNPs modified MIP sensor detection glucose.*

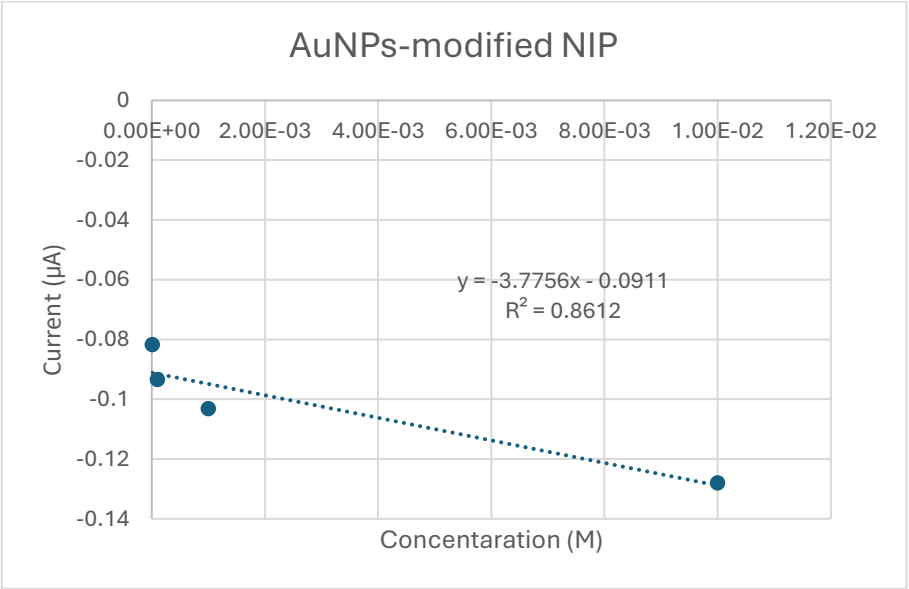


*Figure 22 Calibration curve of AuNPs modified NIP sensor detection glucose.*

The obtained calibration curve used a linear trendline. Therefore, the previously mentioned standard formula was used to calculate the limit of detection (LOD) of the AuNPs-modified MIP sensor from the obtained calibration curve. Table 3 represents the LOD and $R^2$ of the MIP sensor.

*Table 3 LOD of AuNPs-modified sensor.*

| Glucose | Slope | $R^2$ | Standard deviation | LOD (M) |
|---|---|---|---|---|
| **MIP ($10^{-2}$ M to $10^{-5}$ M)** | 0.9028 | 0.9028 | 0.000178195 | **$5.6 \cdot 10^{-4}$ M** |
| **NIP ($10^{-2}$ M to $10^{-5}$ M)** | 3.7756 | 0.8612 | $4.86487 \cdot 10^{-4}$ | **$3.8 \cdot 10^{-4}$ M** |

The LOD of the AuNPs-modified sensor indicates that AuNPs decreased the sensor's functionality compared to the chitosan-modified sensor. Also, comparing the MIP sensor with the corresponding NIP sensor, the LOD of the sensor without cavities has a lower value, which indicates the inefficiency of MIPs. Besides, its lower $R^2$ value than the chitosan-modified sensor means it is less reliable than the chitosan-modified sensor. For further analysis, the AuNPs-modified sensor was analyzed using cyclic voltammetric techniques.

**Cyclic Voltammetry**

Once the electrodeposition is successful, the sensor can be measured in potentiometric or cyclic voltammetric. Figure 23 is the voltammogram of the AuNPs-modified sensors NIP and MIP towards $10^{-2}$ M. Based on this voltammogram, the intensity of current obtained in the MIP sensor has a slightly higher response in the cathodic and anodic region than that of the NIP sensor. This is a possible indication of the holes created by the template molecule in the MIP sensor.

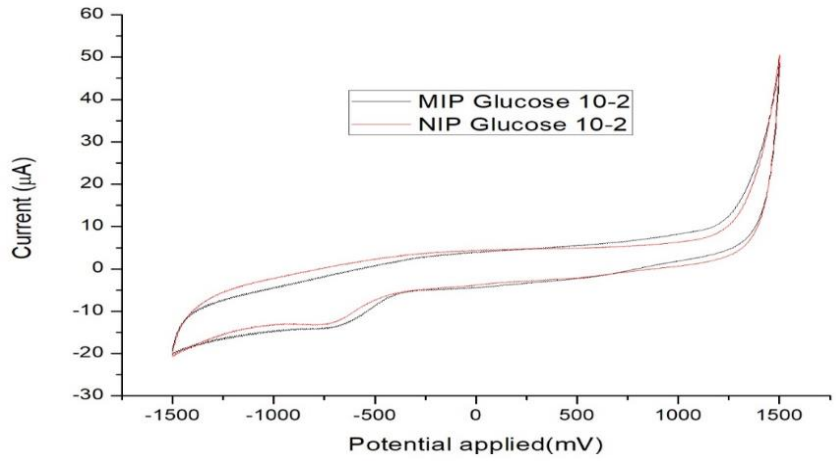


*Figure 23 Cyclic voltammetric response of the MIP and NIP towards a $10^{-2}$ M glucose solution.*

The voltammetric response of the AuNPs-modified sensor to increasing the concentration of glucose solution from $10^{-5}$ to $10^{-2}$ M is analyzed. The maximum reduction at the peak is observed at -715 V. From this peak, the calibration curve is obtained, and Figures 24 and 25 represent this calibration curve.

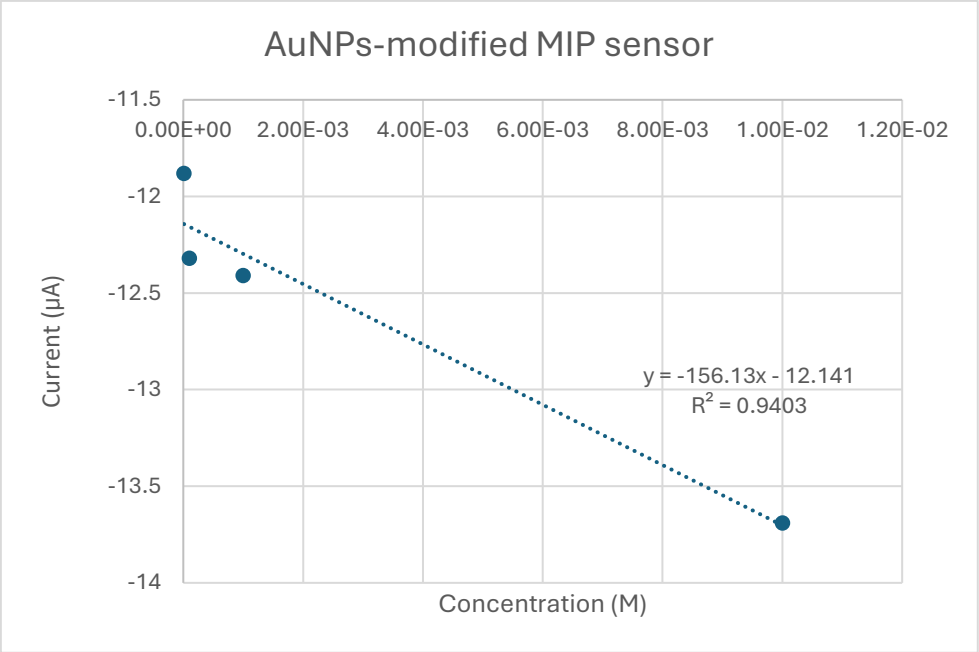


*Figure 24 Calibration curve of AuNPs-modified MIP sensor.*

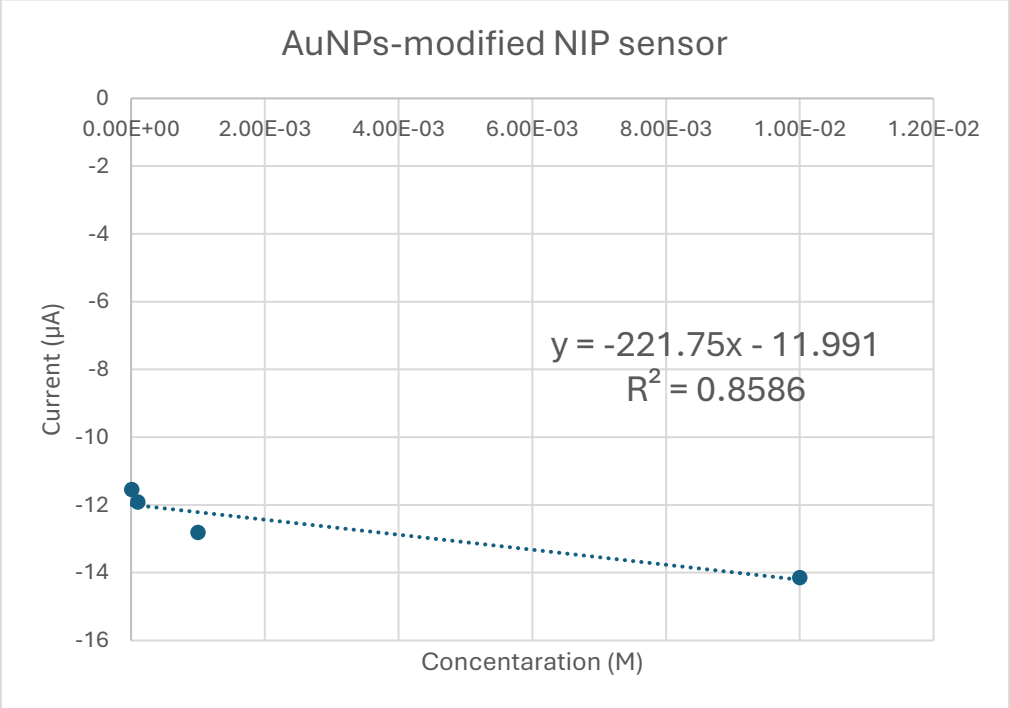


*Figure 25 Calibration curve of AuNPs modified NIP sensor detection glucose.*

The calibration curve and standard deviation from the sensors' blank (0.1 M KCl) help calculate the LOD of a cyclic voltammetric AuNPs-modified MIP sensor. To calculate the previously mentioned formula for LOD is used. The resulting calculation created Table 4.

*Table 4 LOD of AuNPs-modified MIP sensor*

| Glucose | Slope | $R^2$ | Standard deviation | LOD (M) |
|---|---|---|---|---|
| **MIP ($10^{-2}$ M to $10^{-5}$ M)** | 156.13 | 0.9403 | 0.255611 | **$4.9 \cdot 10^{-3}$ M** |
| **NIP ($10^{-2}$ M to $10^{-5}$ M)** | 221.75 | 0.8586 | 0.380344 | **$5.1 \cdot 10^{-3}$ M** |

The analysis of the AuNPs-modified MIP sensor in cyclic voltammetry suggested that cyclic voltammetry gives much less response than the potentiometric sensor based on the LOD of the sensors. Based on LOD, the sensor with captives has much more efficiency than the sensor without cavities. Adding AuNPs also helps improve the LOD of the AuNPs-modified MIP sensor more than the standard chitosan-modified MIP sensor in the case of cyclic voltammetry response.

### 5.2.3. Sensing behavior of the AgNPs modified MIP for Glucose detection.

As explained before, the metallic nanoparticles (AuNPs and AgNPs) were used in the development of the MIP sensors. The aqueous silver nanoparticles (AgNPs) are prepared by the reduction of $AgNO_3$ with $NaBH_4$. Like the fabrication of AuNPs-modified MIP sensors, the AgNPs are mixed with glucose chitosan in a volume ratio of 1:2 AgNPs: CS.

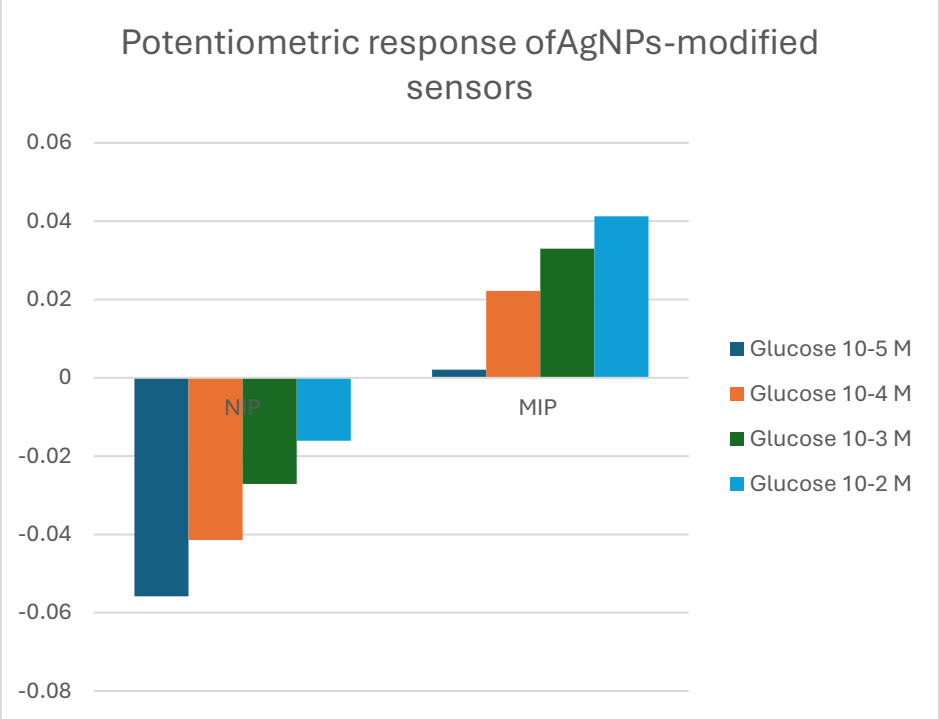


*Figure 26 Potentiometric response of AgNPs modified NIP and MIP towards glucose solution.*

It is observed that the intensity of current increases with concentration; in the case of MIP to positive values, the sensor's capability to detect glucose molecules is demonstrated. At the same time, NIP sensor values are denoted negatively. For further analysis, calibration is obtained from these potentiometric responses.

Figure 27 shows the calibration curve obtained for the AgNPs-modified MIP sensor to detect, and Figure 28 represents the calibration curve obtained for the AgNPs-modified NIP sensor. This calibration curve is obtained from a concentration of glucose solution ranging from $10^{-4}$ to $10^{-5}$ M.

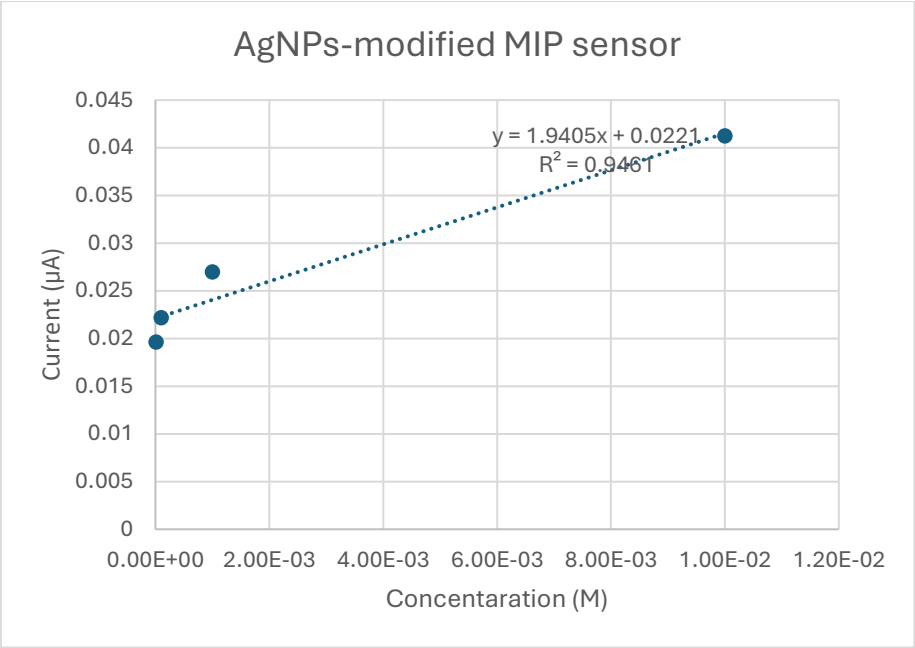


*Figure 27 Calibration curve of AgNPs-modified MIP sensor.*

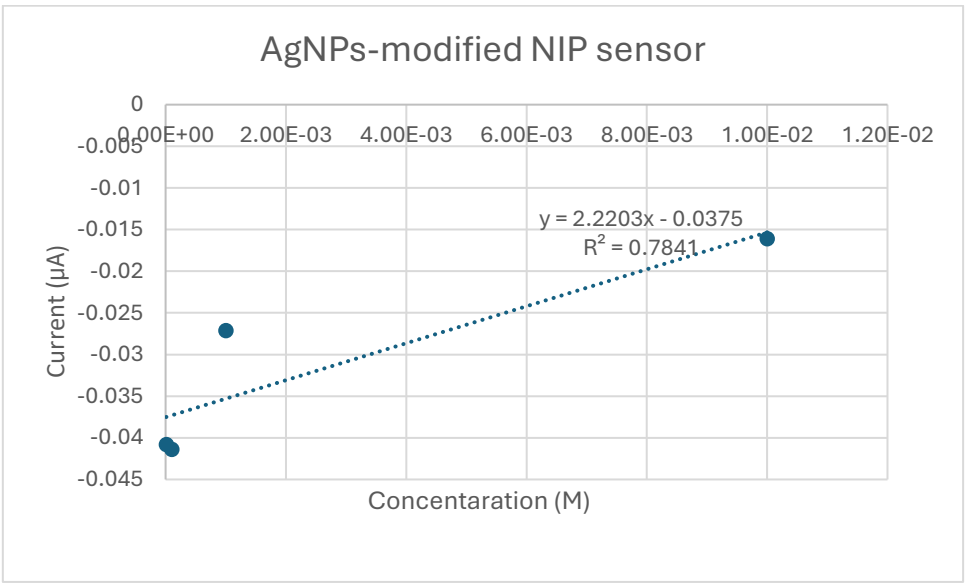


*Figure 28 Calibration curve of AuNPs modified NIP sensor detection glucose.*

Based on the calibration curve obtained, the LOD of MIP and MIP is calculated using the previously mentioned formula. As a result, Table 5 is obtained. The LOD was calculated using the standard deviation of the sensor blank (0.1 M KCL) and the calibration slope.

*Table 5 LOD of AgNPs-modified MIP sensor.*

| Glucose | Slope | $R^2$ | Standard deviation | LOD (M) |
|---|---|---|---|---|
| **MIP ($10^{-2}$ to $10^{-5}$ M)** | 1.9405 | **0.9339** | 0.000131788 | **$2.03 \cdot 10^{-4}$ M** |
| **NIP ($10^{-2}$ to $10^{-5}$ M)** | 2.2203 | **0.7841** | 0.006898732 | **$9.1 \cdot 10^{-3}$ M** |

The obtained LOD of the AgNPs-modified MIP sensor in the potentiometric technique is $2{,}03 \cdot 10^{-4}$ M. This is the lowest limit of detection of the other two sensors, suggesting that AgNPs increase the performance of the MIP sensor.

**Cyclic Voltammetry**

After the successful electrodeposition, the sensor can be measured using potentiometric or cyclic voltammetric methods. Figure 29 is the voltammogram of the AgNPs-modified sensor NIP and MIP towards $10^{-2}$ M. Based on this figure, in $10^{-2}$ M concentration, MIP has a higher response than NIP. Therefore, this voltammogram identifies the created hole in the MIP sensor.

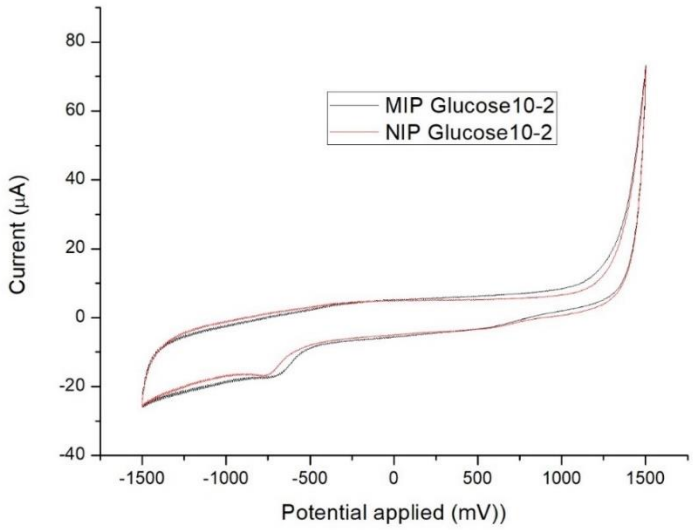


*Figure 29 Cyclic voltammetric response of the MIP and NIP towards a $10^{-2}$ M glucose solution.*

The next step is to construct the calibration curve of the MIP sensor, and it is possible to do so by taking the response MIP sensor from the concentration of glucose solution varies $10^{-2}$ to $10^{-5}$ M. Figure 30 is obtained calibration curve of the AgNPs-modified MIP sensor, and Figure 31 is the calibration curve of the AgNPs-modified NIP sensor.

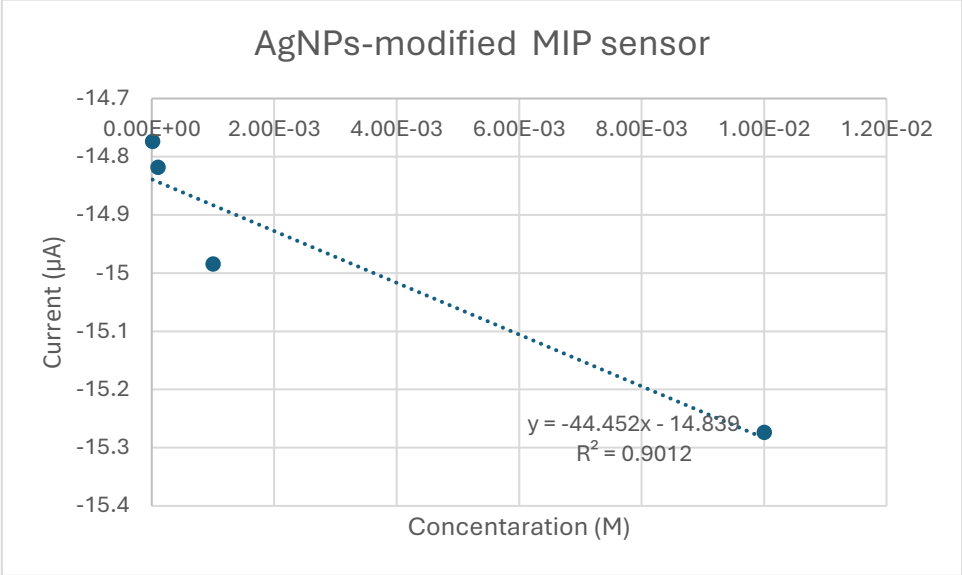


*Figure 30 Calibration curve of AgNPs-modified MIP sensor.*

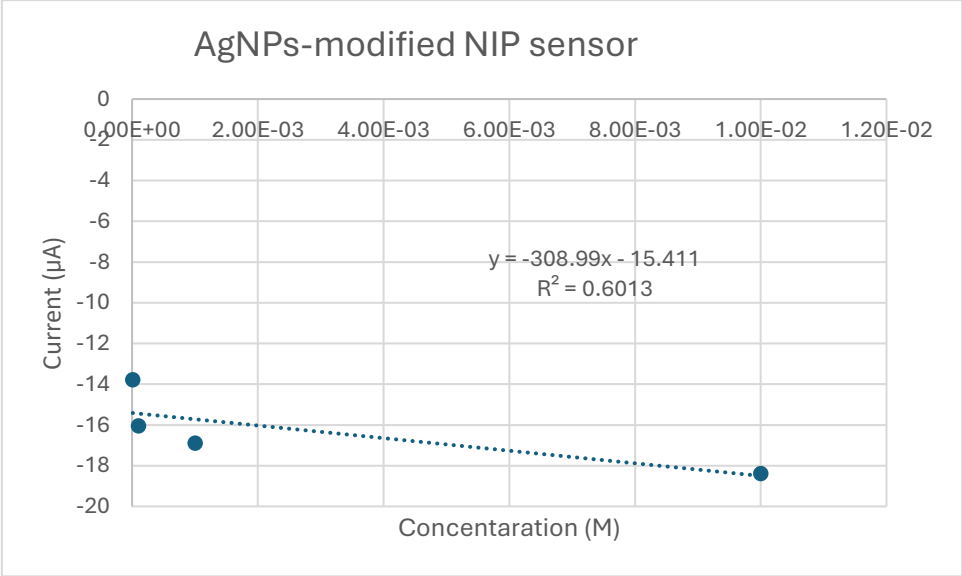


*Figure 31 Calibration curve of AgNPs modified NIP sensor detection glucose.*

Based on the calibration curve and standard deviation of the KCl response the limit of detection (LOD) of the MIP sensor is calculated. Table 6 is the calculated LOD of the cyclic voltammetric AgNPs-modified MIP sensor.

*Table 6 LOD of AgNPs-modified MIP sensor.*

| Glucose | Slope | $R^2$ | Standard deviation | LOD (M) |
|---|---|---|---|---|
| **MIP ($10^{-2}$ M to $10^{-5}$ M)** | 44.452 | **0.9012** | 0.0760043 | **$5.1 \cdot 10^{-3}$ M** |
| **NIP ($10^{-2}$ M to $10^{-5}$ M)** | 308.99 | **0.6013** | 0.1693026 | **$1.6 \cdot 10^{-3}$ M** |

Based on the LOD of the cyclic voltammetric response of the AgNPs-modified MIP sensor is lower than $5.1 \cdot 10^{-3}$. Compared with LOD from potentiometry, cyclic voltammetry is less effective. The corresponding NIP sensor shows lower LOD, but its $R^2$ is very low compared to the MIP sensor. The overall comparison between cyclic voltammetry and the potentiometry response of sensors suggests the potentiometric readout method is more effective than the cyclic voltammetry. The LOD remains above $10^{-3}$ M in cyclic voltammetry, while in potentiometry, the MIP sensor can have an LOD between $10^{-3}$ and $10^{-4}$ M. This indicates that the potentiometric technique works appropriately to detect glucose. By comparing the MIP sensor with the potentiometric response, the most effective sensor is the AgNPs-modified MIP, the LOD of this sensor is $2.03 \cdot 10^{-4}$ M. Even though the AuNPs-modified MIP sensor can detect glucose, compared to chitosan-modified and AgNPs-modified sensors, the AuNPs is not practical.

### 5.2.4. MIP sensor for lactose detection.

Once the sensor for detecting glucose was completed, more complex structure sugar detection possibilities were started. On that basis, the detection of the disaccharide molecule lactose was investigated. For the development of the lactose MIP sensor, the same protocol was followed as for the glucose MIP sensor. Therefore, chitosan polymer is used in the development of MIP sensors.

### 5.2.5. Sensing behavior of the chitosan-modified MIP for lactose detection.

After electrodeposition, the chitosan-modified MIP sensor removes the template molecule using an eluent. Then, this MIP sensor is read out using either potentiometry or cyclic voltammetry. Figure 32 represents the potentiometric response of the MIP and NIP sensors in a standard solution of lactose, which varies from $10^{-2}$ to $10^{-5}$ M.

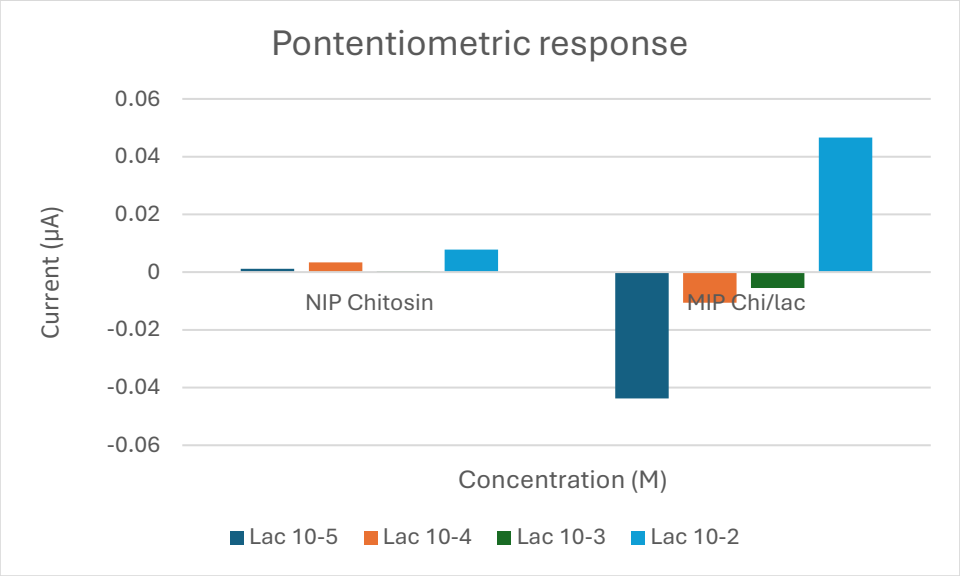


*Figure 32 Potentiometric response of chitosan-modified NIP and MIP towards lactose solution.*

The obtained potentiometric response of the MIP sensor shows a significant incline in the signal between the concentrations $10^{-3}$ and $10^{-2}$. A comparison between the NIP and MIP sensor

responses is nonlinear. The MIP sensor's current intensity appeared negative in value, which suggests the possibility of no captives/hole formation or that the lactose molecules are unable to bind with specific captives in the MIP sensor. For further clarification, cyclic voltammetric responses of chitosan modified sensor is measured.

**Cyclic Voltammetry**

As in the potentiometry, the chitosan-modified MIP sensor is measured by cyclic voltammetric methods. Figure 33 shows the cyclic voltammetric response of the MIP and NIP sensors toward a $10^{-2}$ M lactose solution. It's clear that the NIP sensor has a higher response than the MIP sensor. This confirms that chitosan-modified MIP sensors are not working for the detection of lactose.

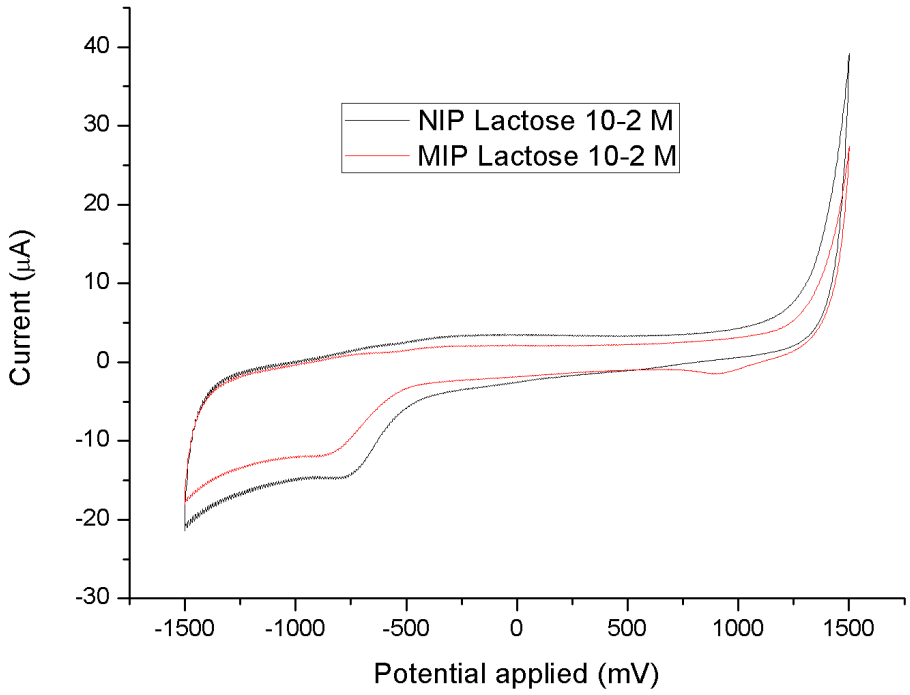


*Figure 33 Cyclic voltammetric response of the MIP and NIP towards a $10^{-2}$ M lactose solution.*

By comparing the potentiometric and cyclic voltammetric response of chitosan, the MIP sensor for the detection of lactose is not working as expected. In potentiometric response, the MIP shows negative and positive potential. It indicates the possible inactivity of the MIP sensor. Further investigation with cyclic voltammetric confirms that MIPs are inactive by showing a higher NIP

signal than MIPs. To conclude this recurring phenomenon the nanoparticle-modified sensors for lactose detection are investigated.

### 5.2.6. Sensing behavior of the AgNPs-modified MIP for lactose detection.

AgNPs-modified MIP sensors were developed to investigate the application of nanoparticles in the detection of lactose in MIP sensors. After the unsuccessful fabrication of the chitosan-modified MIP sensor, an investigation was carried out in the AgNPs-modified MIP sensor to detect lactose. The electrodeposition is carried out with AgNPs chitosan matrix and lactose for these purposes. After successful electrodeposition and elution, this sensor is measured by cyclic voltammetric. Figure 34 is the obtained voltammogram of MIP and NIP response of $10^{-4}$ M lactose.

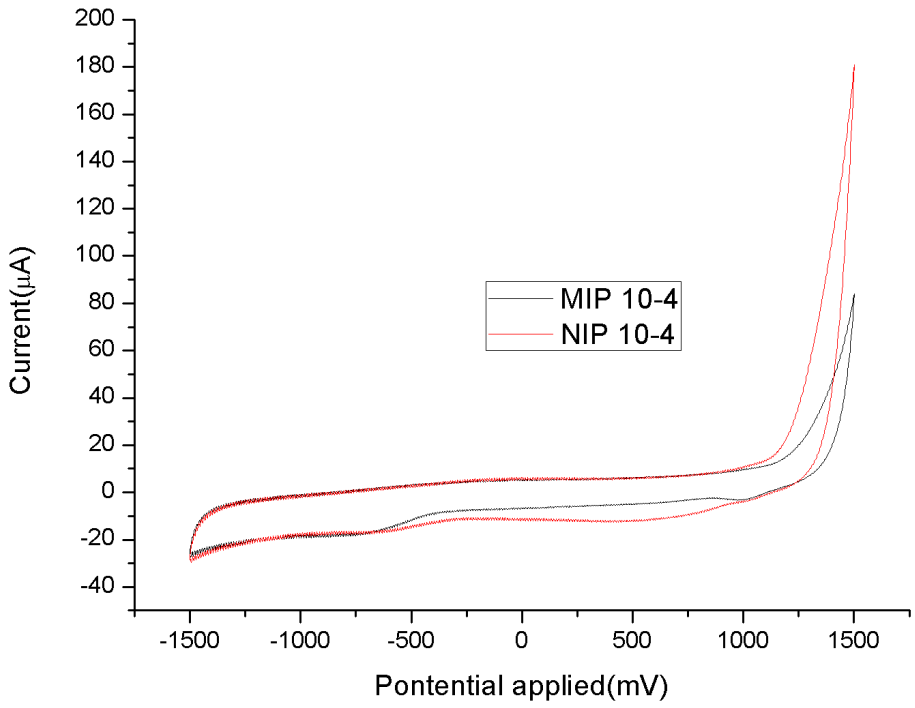


*Figure 34 Cyclic voltammetric response of the AgNPs-modified MIP and NIP towards a 10-2 M lactose solution.*

The analyzed MIP and NIP response to lactose shows that NIP has a higher signal than MIP. This determines that the fabricated sensor is not working correctly. Repeating the same signal as the chitosan-modified sensor proved that it is a recurring event in detecting lactose sensors.

Developing a lactose sensor with the same protocols as the glucose MIP sensor is ineffective. It is possible to achieve versatility in developing sensors by using molecularly imprinted chitosan. Each template molecule needs a different developing protocol rather than switching the template molecules.

# 6. Conclusion

The results of the investigation give a significant conclusion to the experiment. These are the following:

- The method for developing the chitosan-modified MIP sensor to detect glucose was prosperous compared to lactose.
- The potentiometric method is more effective than the cyclic voltammetric method for glucose detection.
- The addition of nanoparticles intensified MIP response, but AgNPs were the only effective. The AgNPs-modified MIP sensor is the most efficient for detecting glucose with an LOD of $2.03 \cdot 10^{-4}$ M.

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