BUSE UĞUR

DEVELOPMENT OF A PLASMONIC SENSOR FOR A CHEMOTHERAPEUTIC AGENT CABAZITAXEL

PhD THESIS FALL,2023

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

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Fall, 2023

Approval

We certify that we have read the thesis submitted by Buse UĞUR titled **"Development of a Plasmonic Sensor for a Chemotherapeutic Agent Cabazitaxel"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctoral of Applied Sciences.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

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Buse Uğur

Abstract

Development of a Plasmonic Sensor for a Chemotherapeutic Agent Cabazitaxel

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PhD, Department of Biomedical Engineering
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Drug dosage is a crucial subject in both human and animal treatment. Administrating less drug dosage may prevent treatment or make it less effective, and high drug dosage may cause a heightened risk of adverse effects, or in some cases, it can cost a patient's life. Also, even when the dosage is administered carefully, metabolic differences may cause a different effect on a patient. Because of these subjects, monitoring drug dosage in the body is a critical and significant desire in the health industry. Within the scope of this study, a reusable surface plasmon resonance (SPR) chip with fast response, high selectivity, and no pretreatment is produced for the chemotherapeutic agent cabazitaxel. Cabazitaxel imprinted nanofilm was synthesized on the sensor chip surface, characterized by atomic force microscopy, ellipsometer, and contact angle measurements. Standard cabazitaxel solution and an artificial plasma sample were used for the kinetic analysis. Docetaxel, methylprednisolone, and dexamethasone were analyzed for their selectivity experiment. Besides, the repeatability and storage durability of the sensor was also evaluated. As a result of the adsorption studies, the limit of detection and limit of quantitation values were found to be 0.012 µg/mL and 0.036 µg/mL, respectively. High-performance liquid chromatography analysis was used to validate the response of the cabazitaxel imprinted sensor.

Keywords: Cabazitaxel, surface plasmon resonance, molecularly imprinted polymer, drug detection, plasmonic sensor.

Table of Contents

CHAPTER III

CHAPTER IV

CHAPTER V

List of Tables

List of Figures

List of Abbreviations

CRPC:	Castration-resistant prostate cancer
CTX:	Cabazitaxel
DTX:	Docetaxel
SPR:	Surface plasmon resonance
MIP:	Molecularly imprinted polymers
HPLC:	High-performance liquid chromatography
AFM:	Atomic force microscopy
CA:	Contact angle
DHT:	Dihydrotestosterone
PSA:	Prostate Specific Antigen
KT:	Chemotherapy
iv:	Intravenous
IA:	Intra-arterial
IP:	Intraperitoneal
FDA:	The American Food and Drug Administration
IR:	Irinotecan
Db:	Dacarbazine
kDa:	Kilodalton
MAA:	Methacrylic acid
AA:	Acrylic acid
EGDMA:	Ethylene glycol dimethacrylate
ELISA:	Enzyme-linked immunosorbent assay
QCM:	Quartz crystal microbalance
nm:	Nano meter
mg/mL:	Miligram per mililiter
HEMA:	2-hydroxyethyl methacrylate
MIP	Molecularly imprinted polymer
NIP:	Molecularly Non-imprinted polymer
µg/mL:	Micro-gram per mili-Liter
LOD:	Limit of detection

LOQ:	Limit of quantification
DEX:	Dexmethasone
RSD:	Relative standard deviation
m:	Slope
nM:	Nano molar
SD:	Standard deviation
R^2:	Correlation coefficient

CHAPTER I
Introduction

In the European Union (EU) countries, cancer is the leading cause of death among people younger than 65 (Dyba et al.,2021). Breast, colon, lung, and prostate cancers account for 50% of all cancer diagnoses. Prostate cancer (22.2% of all males, lung, 14.8%, colorectum,13.2%, bladder, 7.3%) is the most prevalent among men (Dyba et al.,2021).

In Europe, prostate cancer will rank as the fourth most prevalent cancer in 2020. The introduction of PSA testing in the early to mid-1990s, which is mainly responsible for the increase in prostate cancer incidence rates (Arnold et al., 2015), led to a rapid increase in the detection of prostate cancers in their early stages during the early to mid-1990s.

Several solid cancers, including the prostate, may be linked to chronic inflammation. Prostate carcinogenesis may be aided by oxidative stress and reactive oxygen species produced from inflammation. The discovery of a urinary microbiome indicates that the prostate may regularly be exposed to a wide range of microorganisms, which could contribute to an inflammatory microenvironment(Sfanos et al.,2018). However, it has not yet been determined which of the many infectious agents found in prostate tissue samples or prostate secretions is responsible for damaging or inducing inflammation in the prostate.

Castration-resistant prostate cancer (CRPC) accounts for the majority of deaths (Wang et al.,2007). Several treatment options for CRPC are currently in use, including immunotherapy, radiotherapy, chemotherapy, vaccine therapy, and experimental therapies(Gillessen et al.,2015) (Parker et al.,2020) (Fitzpatrick et al.,2014). Ongoing clinical trials have shown that chemotherapy is an effective treatment option. Docetaxel (DTX), cabazitaxel (CTX), estramustine, and mitoxantrone are examples of common chemotherapeutic agents (Song et al., 2018). CTX inhibits tumor cell mitosis and prevents androgen receptor translocation into the nucleus. Recent evaluations have compared the reduced dose of CTX to the currently approved dose (25 mg/m^2 Body Surface Area) (Karavelioglu et al.,2016).

Some chemotherapeutic agents can cause severe neurological adverse

effects, lowering the standard of living and limiting the amount that can be administered. Human tumors that are relatively resistant to chemotherapy and patients with advanced prostate cancer despite DTX treatment have shown promising results with the next-generation CTX (Karavelioglu et al.,2016). For example, CTX can cross the blood-brain barrier, whereas DTX and paclitaxel have low absorption potentials (Virugnaud et al.,2019). Intriguingly, the neurotoxic effect of CTX was found to be lower than that of other taxanes such as DTX and paclitaxel.

The amount of medicine or drug is taken into the body is determined with toxicology screening tests, where the life quality of patients was demonstrated to be severely affected by those toxicities (Oudard et al.,2017) (Antonarakis et al.,2017) (Al-Batran et al.,2015). Such an analysis is done with instrumental methods for drugs/medicines (Mohan et al.,2017). Using these methods in the analysis takes a long time, and it is laborious, which is not a wanted feature because late analysis means late diagnosis and late treatment, which may result in the patient's death or seriously harm the patient. Various sensors have been developed in the last decade to address this issue (Zhang et al., 2015). Sensors are small devices that can sense their environment and use them for chemical analysis (Tang et al.,2014) (Marino et al.,2015). Their flexible structure and small size make them an alternative to classic laboratory analysis equipment. Also, making them reusable or disposable is possible, the desired feature in some cases.

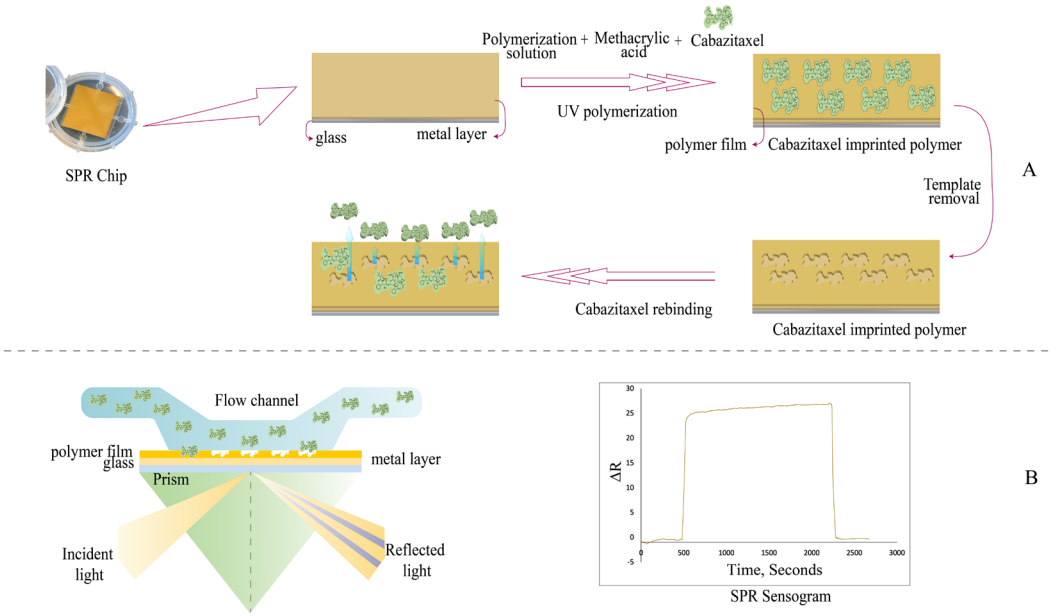
Optical sensors can detect many biological and chemical substances directly, label-free, and in real-time, which have significant advantages over traditional analytical methods. Their advantages include accuracy, easy-to-use, low cost, high specificity, and sensitivity (De Bono et al., 2010) (Kurç and Türkmen, 2022). Surface plasmon resonance (SPR) is one of the most prevalent optical sensor sub-classes. Electrons in the conduction band oscillate collectively in resonance with the oscillating electric field of the incident light, causing SPR to occur (Li and Zhang,2017). SPR's lack of a label makes it ideal for studying molecular interactions rapidly, precisely, and sensitively (Genslein et al.,2016). SPR sensors have a wide range of applications, including diagnosing, disease surveillance, enzyme-linked immunosorbent assay, diagnostic and therapeutic analysis, biomedical, processing industry, animal health, environmental control of pollution, and agriculture applications (Safran et al., 2021) (Saylan et al.,2022) (Çimen et

al.,2022). Applying surface functionalization to a sensor can enhance its specificity against target molecules. In the molecular imprinting technique, macromolecules with target molecule-specific recognition sites can be synthesized (Derazshamshir et al.,2021) (Shama et al.,2022). Molecularly imprinted polymers (MIP) have numerous excellent characteristics, including stable chemical, physical, and mechanical properties, high pressure and temperature resistance, strong resistance to acids and alkalis, simple production, long-lasting performance, reuse, and recycling (Derazshamshir et al.,2021).

SPR-based sensors are used to simultaneously directly measure interactions between biomolecules without any marking, and the scheme of SPR-based sensor is demonstrated in Figure 1A. This technology is based on detecting minute changes in the refractive index of thin metal (Au, Ag) films brought about by the interaction of target compounds with a specific transducer via a change in the resonance angle. (Türkmen et al.,2021). The molecular imprinting method is based on making template-specific cavities in a cross-linked polymer matrix, as seen in Figure 1B. These cavities can detect the target molecule's size and shape. Any desorption technique that removes the template molecule reveals functional monomer groups in the right places, forming a structural binding site for the target molecule.

Figure 1:

A) CTX template molecular imprinting on the SPR chip's surface,
B) The working principle of SPR-based sensor



Here, CTX imprinted methacrylic acid-ethylene glycol dimethylacrylate-hydroxyethyl methacrylate (CTX MIP) sensor was synthesized on the SPR chip surface as a synthetic receptor for CTX. Kinetic studies were carried out after the preparation and characterization of the polymeric nanofilm. CTX samples prepared at different concentrations were applied to the SPR sensor system, the binding kinetic parameters were evaluated, and the sensing performance was measured. Additionally, the CTX spiked artificial plasma sample was analyzed then the selectivity, and reusability experiments were performed. HPLC analysis was used to validate the response of CTX MIP sensor**.**

Statement of the Problem

Drug dosage is a crucial subject in both human and animal treatment. Administrating less drug dosage may prevent treatment or make it less effective, and high drug dosage may cause a heightened risk of adverse effects, or in some cases, it can cost patients life. Also, even when the dosage is administrated carefully, metabolic differences may cause a different effect on a patient. Because of these subjects, monitoring drug dosage in the body is a critical and significant desire in the health industry.

Purpose of the Study

The objective of this study was to create a surface plasmon resonance (SPR) sensor chip for the chemotherapeutic medication cabazitaxel, which is used to treat many forms of prostate cancer. The sensor we developed are reusable, fast reacting, easy to use and requires no pretreatment to detect cabazitaxel.

Research Questions/Hypotheses

Generally, the main research hypothesis can be determined as follows:

- Why do I need this study?
- How it help the specialists ?
- Does it play important role for people health ?
- What will be the detection limit when the SPR chip surface is modified with MIP?
- What about the repeatability of the sensorchip?
- What will be the selectivity of the chip cabazitaxel detection ?
- Will the chip be able to provide sufficient sensitivity for cabazitaxel determination ?

Significance of the Study

Drug dosage is a crucial subject in both human and animal treatment. Administrating less drug dosage may prevent treatment or make it less effective, and high drug dosage may cause a heightened risk of adverse effects, or in some cases, it can cost a patient's life. Also, even when the dosage is administered carefully, metabolic differences may cause a different effect on a patient. Because of these subjects, monitoring drug dosage in the body is a critical and significant desire in the health industryIn the context of this study, a reusable surface plasmon resonance (SPR) chip is created for the chemotherapeutic drug cabazitaxel that has a quick reaction, good selectivity, and requires no precondition..

This thesis is an example of interdisciplinary study which combines biomedical engineering, nanotechnology and medical diagnosis.

Limitations

HPLC is the only method which is used to analyse Cabazitaxel and the kits are expensive and not available in Cyprus. and our method which is SPR that we

aim to use for analysing Cabazitaxel is not found in Cyprus. the studies should be done in Turkey.

Definition of Terms

The following procedures apply to the key concepts in this work:

- Cabazitaxel: is a chemotherapeutic agents which inhibits tumor cell mitosis and prevents androgen receptor translocation into the nucleus
- Surface plasmon resonance (SPR): is one of the most prevalent optical sensor sub-classes. Electrons in the conduction band oscillate collectively in resonance with the oscillating electric field of the incident light, causing SPR to occur. SPR's lack of a label makes it ideal for studying molecular interactions rapidly, precisely, and sensitively
- Molecularly imprinted polymer MIP: refers to a class of synthetic materials that are used as differentiating features in sensor design because they are more thermally stable, selective, and cost - effective than biological receptors.Atomic force microscopy (AFM): To investigate the depth of the surface, an ambient AFM was used in tapping mode. It can take measurements with very high resolution from the AFM free cantilever interferometer feature.
- Ellipsometer: is the refraction and reflection of light from a material. It measures the change in polarization.
- Contact angle (CA):

CHAPTER II
Theoretical Framework

Cancer

The term cancer was used for the first time by Hippocrates, and was named carcinos and carsinoma due to the solid tumor section looking like a crab.

Uncontrolled cell division and spread are two characteristics of the condition known as cancer (Akdemir and Birol,2003). According to the 2021 data of the World Health Organisation, cancer types with the highest incidence and leading to death are lung, breast, colorectal, prostate and skin cancers (Vrignaud et al.,2013). About ten thousand mutations occur daily in our body cells, and these Mutations are regularly destroyed by our immune cells. In cases where the efficiency of the Immunity cells are impaired, these cells cannot be destroyed and they multiply in such a way that they invade the tissue they belong to and even invade neighboring tissues. They metastasize to other parts of the body through the blood and lymph circulation (Villanueva,2015).Cancer cells proliferate rapidly in the areas they occupy, they increase the carbon dioxide rate and accordingly hypoxia occurs (Abdollahi,2010). In hypoxia environment, In the presence of cancer cells and oncogenes, angiogenesis promotes vascular formation. They form new capillaries from existing vessels by secreting factors. This is called angiogenesis. When the genetic material of normal cells is damaged DNA repair mechanisms is activated and the repair of DNA takes place. If the damage is not repaired a programmed cell death starts to occur and this is called apoptosis (Carson and Riberio,1993). On the other hand, the DNA of cancer cells cannot be repaired by apoptosis and tumor formation occurs with uncontrolled proliferation. According to scientists 10 key features of cancer have been identified.

Common Cancer Types

Cancer cells are classified according to the tissue-organ type from which they originate and their microscopic images. Although there are nearly 100 types of cancer, some types are seen very frequently . The World Health Organization's statistical data from 2021 show that prostate, lung, breast, colon, uterine, urinary system, and melanoma skin cancers have the greatest incidence rates and leading causes of death from cancer.

Prostate Cancer

The prostate is tissue located in front of the rectum, under the bladder, found only in men. The task of this gland is to nourish and protect the sperm during the release of sperm from the testicles producing and secreting seminal fluid. Prostate cancer (PK), is defined as enlargement of prostate gland cells and enlargement in the organ volume with loss of division control. Prostate cancer is the sixth most common cancer worldwide and It is the most common type of cancer in men after skin cancer (Gronberg,2003).

Prostate gland peripheral zone, central zone, transitional zone, fibromuscular zone consists of five regions, including the preprostatic sphincteric zone (Lowsley,1912). Of these The peripheral zone makes up most of the prostate and most prostate cancers are located in this region starts. The central zone is the part that surrounds the semen duct. 2.5% of prostate cancer is central cancer originating from the zone and is the more aggressive type that can easily penetrate more into the seminal vesicle (Grönberg,2003). The transitional zone is the portion of the prostate that surrounds the urethra. 10-20% of it comes out of this zone and benign prostatic enlargement occurs in this part. The structure of the fibromuscular zone consists of muscle and connective tissue and it does not contain glandular structure.

Preprostatic Sphincteric zone: It is the mechanism that allows the semen to be thrown forward (Lowsley,1912).

The Impact of Androgen on the Development of Prostate Cancer

Androgen known as male hormone is effective in the development and progression of prostate cancer (Salamon et al.,2017). Androgens are secreted from the cortex of the adrenal gland. They allow the synthesis of testosterone in the testicles. Synthesized testosterone pass into prostate cells by diffusion where they are converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase. DHTs bind to androgen receptors and activate it (Abbasoğlu,2018). After the androgen receptor that is free in the cyptoplasim is activated, it goes to the cell nucleus and binds to the androgen receptor site. As a result of this, cells responsible for the proliferation of PSA genes become active. Excess of activation of androgen

receptors are responsible for the development of neoplasia in prostate cells (Fiazi et al.,2019).

Prostate Specific Antigen (PSA)

Prostate cells release PSA, a serine protease glycoprotein that softens sperm (Chi et al.,2018). PSA is found in high concentration in seminal fluid, but very low in serum. PSA blood passes into circulation in the prostate in the event of structural and functional deterioration. If there is neoplasia formation in the prostate, PSA is synthesized in excess (Swami et al.,2020). Only 5-20% of PSA is free in circulation, most of it binds to α-1-antichymotrypsin and α-2-macroglobulin inhibitors in serum. In cases of Prostate cancer the amounts of free PSA and PSA bound to α-1-antichymotrypsin in serum in increase (James et al.,2017).

The Role of Genetic Factors in the Pathogenesis of Prostate Cancer

The structure of normal prostate cells is disrupted and transformed into metastatic prostate cells. Oncogenes, tumor suppressor genes, angiogenesis, DNA repair genes disorders, genetic instability can be effective (Hoyle et al.,2019). Studies show that Chromosomes with structural defects such as insertions and deletions NKX3.1, PTEN and DNA have been obsereved to have repair genes such as MYC (Chi et al.,2019). However, prostate cancer development Mutations in p53, one of the suppressor genes, are very rare (Agarwal et al.,2019). Abnormal DNA methylation, rearrangement of chromatin and microRNA epigenetic changes also play an important role in the development of prostate cancer (Chi et al.,2021). Genes of prostate cancer cells have hypermethylation to suppress the promoter region and the formation of neoplasia. This modification causes silencing of DNA repair genes, hormone Tumor involved in impaired signal transduction, cell cycle checkpoints, and apoptosis causes loss of function in suppressor genes. In addition to these, activation of oncogenes including c-MYC, HRAS, and hypomethylation of chromosomal stability is hypothesized to contribute to the etiology of prostate cancer by impairing it. (Dawis et al.,2019).

Cancer of the prostate treatment

Surgical operation, radiotherapy, hormone therapy and chemotherapy are applied in the treatment of PC (Davis et al.,2019). In the treatment of the disease,

docetaxel and Cabazitaxel is the most commonly used chemotherapy drug. The Chemotherapy drug Docetaxel combined with steroid-based prednisone Chemotherapy is usually given first for treatment (Armstrong et al.,2019). If this medicine is not effective Cabazitaxel is usually the next chemotherapy drug used. Cabazitaxel anti-neoplastic acts by disrupting the microtubular network balance and cell cycle(Shore et al., 2020).

Melanoma Cancer

Melanoma cancer is the deadliest of all skin cancers (Shore et al.,2020). Melanoma, consists of melanocyte cells that give color to the skin with the production of melanin pigment (Armstrong et al.,2019).Skin cancers first begin in the epidermis. epidermis from outside to inside consist of three main cell types, namely right squamous cells, basal cells, and melanocytes. Squamous cells are the outermost group of cells, they pave the inner cells of the skin. Basal cells lie just below the squamous cells and are responsible in forming new skin. (Shore et al.,2020). Both types of skin cancers start mostly at the head and neck which is the most sun-exposed parts. These cancers are associated with exposure to intense sunlight (Berthold et al.,2008). Melanocytes are in the lowest layer of the epidermis which produce melanin pigment, which gives the skin its normal color (Berthold et al.,2008). Melanoma neural cleft-derived melanocytes (skin, mucous membranes, eyes and central nervous system) and melanocytes originates from the nevus cells formed as a result of the change (Eisenberger et al.,2017). Although melanomas can occur in any part of the body, it is often found on the chest and back of the body in men (Oudard et al.,2017).

Molecular Mechanism of Melanoma Cancer

As in all cancer types, melanoma cancer also has mutations in protooncogenes which becomes oncogene, resulting in loss of activity of tumor suppressor genes. While oncogenes NRAS, BRAF and KIT are activated in melanomagenesis, tumor the suppressor genes p53, CDKN2A and PTEN are inactivated (de Bono et al.,2011). Also the development of melanoma hypermethylation of tumor suppressor genes and hypomethylation of oncogenes such as epigenetic differences. Germline in the CDKN2A gene, one of the negative regulators of the cell cycle, and Positive regulators of the cell cycle are activated by

sporadic mutations. Familial development of melanoma is also frequently encountered (Ryan et al.,2015). Two different proteins called p16INK4a and p14ARF, which slow down the CDKN2A gene cell circulatory cycle and are involved in apoptosis encodes and P16INK4a binds to the CDK4/6 complex and inhibits it (Ryan et al.,2015). Thus, the tumor suppressor protein retinablastoma (RM)-1 is phosphorylated by CDK4/6. Loss of function of the P16INK4a protein is associated with the control of the cell cycle. deterioration occurs and tumor development accelerates (Abida et al.,2019). p14ARF binds to the MDM2 protein breaking it down and provides stabilization to p53. In other words, deletions occurring in p14ARF activate MDM2 which leads to the negative regulation of p53 protein (Hussain et al.,2020). Inactivation of protein p16INK4A and p14ARF results in inactivation of p53 protein and this event is an important step in the development of melanoma (Morris et al.,2021). Melanogenesis c-KIT receptor tyrosine kinase, activates MAPK and PI3KAKT signaling pathways and plays a role in cell proliferation by activating it. MAPK pathway basically consists of RAS, RAF is a signaling pathway in which oncogenes are involved. RAS and RAF mutations are active in many tumors. Early stage mutations in the BRAF gene, which is a member of RAF kinase is common in melanomas (Abida et al.,2020). The RAS family (HRAS, KRAS, and NRAS) transmit the growth signals they receive to target molecules in the cell (Abida et al.,2020). In melanoma many NRAS genes are found (De Bono et al.,2020). A study done on 106 melanoma patients in 2015 in Turkey found 42% BRAF, 15% NRAS, 13% BRAF mutations (Smith et al.,2019). PI3K/AKT signaling involved in cell growth, proliferation and differentiation Irregularities in the pathway are common in melanoma. In 10-30% of melanomas It is observed that the PI3K/AKT pathway remains constantly active as a result of loss of PTEN (Sathianathen et al.,2020). Moreover if there is loss of PTEN accompanied by NRAS and BRAF mutations, melanoma cell lines It is observed to show resistance to inhibitors develops (Sydes et al.,2018). By activation of the MITF oncogene encoding transcription factors responsible for expression of melanogenesis enzymes and differentiation of melanocytes is observed (Rush et al.,2018). MIFT spot in metastatic melanomas mutations have been reported to reduce patient survival (Fizazi et al,2017).

Treatment of Melanoma Cancer

Methods used in treatment of melanoma cancer are surgical removal of cancerous tissue, radiotherapy and chemotherapyThe most popular chemotherapy medication for treating melanoma cancer is dacarbazine. (Sydes et al.,2018). Dacarbazine inhibits DNA synthesis as well as other alkylating agents and destroys the structure of synthesized and healthy DNA structures. It has been determined that Dacarbazine as a drug agent in the treatment of malignant melanoma shrinks tumor by 24% when used (Rush et al.,2020).

Cancer Treatment

Treatment methods of cancer depend on the patient, the patient's gender, age, stage of the cancer and varies depending on the tissue from which it originates. The most common treatment is surgical excision of the cancerous tumour from the body. In addition chemotherapy ,radiotherapy, hormone therapy, immunotherapy and biological methods is applied alone or in combination as supportive treatment (Morris et al.,2018).

Chemotherapy

Chemotherapy (KT) means the treatment of cancer with chemicals and drugs. The drugs used in KT are called "chemotherapeutics". Chemotherapeutics affect cell proliferation by affecting the biochemical processes of the cell. to prevent, to destroy abnormal cells without damaging normal cells, to reduce the possibility of metastasis. surgery, radiotherapy to reduce tumor growth, control pain They are used alone or in combination with therapy methods. chemotherapeutics cancer Depending on the type and drug used, Intravenous (iv), Intra-arterial (IA), Oral pill form, is given to the patient by methods such as intraperitoneal (IP) (Khalaf et al.,2019). Drugs given to the patient in KT are DNA alkylating agents, mitotic inhibitors, topoisomerase inhibitors, anti metabolites, corticosteroids, and anti-tumor antibiotics (Khalaf et al.,2019). Alkylating agents suppress DNA transcription which blocks protein production which stops cell division. When alkylating agents enter the cell Alkyl groups on DNA, they are replaced by hydrogen atoms and have a carcinogenic effect. This agents are successfully used in breast, ovarian, lung cancers, leukemia, multiple myeloma (De Wit et al.,2019). Mitotic Inhibitors bind to microtubules during mitosis and prevents the formation of microtubules. This prevents same family chromosomes from being pulled to

opposite poles. the new cell to be formed is damaged. Thanks to agents such as Docetaxelopoiesis, Paclitaxel, Vinblastine designed to inhibit the formation of many microtubules, many types of cancer such as breast, lung, prostate, can be successfully treated (Freedland et al.,2021). Topoisomerase inhibitors make super-turns in the transcription of DNA. Topoisomerase, which allows the folded threads to unfold and return to their original position make their enzymes dysfunctional. Thus, the transcription of DNA is inhibited and the cell induces apoptosis. Irinotecan and Topotecan inhibit topoisomerase I, while Teniposide and drugs such as Etoposide are used to disrupt the function of topoisomerase II (Maluf et al.,2021). Anti-metabolites are enzymes that are effective in cell proliferation and growth. chemical substances called metabolites, which are responsible for the stimulation and suppression of they provide suppression (Szmulewitz et al., 2018) . Anti-metabolites affect their effects especially in the S Phase of the cell cycle. thereby triggering apoptosis. Purine and pyrimidine analogs, nucleoside analogs, nucleotide analogues and antifolates. For example, the pyrimidine base of direct DNA 5-fluorouracil (5-FU), which disrupts its structure, in the treatment of breast, head and neck, adrenal and gastric cancers used (De Wit et al.,2019).

Corticosteroids are steroid-derived drugs used in the treatment of cancer. Prednisone and Dexamethasone is one of the coticosteroids used in the treatment of cancer. Corticosteroids are used in patients with chronic obstructive pulmonary COPD to reduce the risk of lung cancer (De Bono et al., 2020).

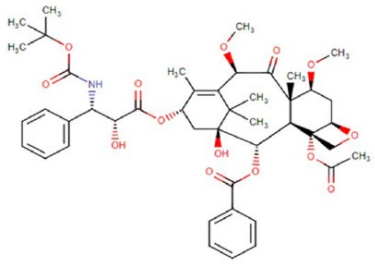
Anti-tumor antibiotics are drugs that suppress DNA and RNA synthesis that slow down cell growth and division in cancer cells. They are widely used in the Leukemia and breast cancer treatment (Agarwal et al., 2020).

Cabazitaxel

Cabazitaxel is obtained from the extraction of the needles of the yew (Taxus baccata) tree. It is a semi-synthetic compound that is a taxoid (Agarwal et al.,2020). In the pharmaceutical market, Cabazitaxel, TXD258, XRP6258, is known by the name JevtanaTM. The American Food and Drug Administration (FDA) approved on 17 June 2010 the treatment of hormone resistant metastatic for prostate cancer and is currently oftenly used in the treatment of prostate cancer. Its molecular weight is 894 Da, its structural it is C45H57NO14. (Fig. 2) (Petrylak et al.,2020)

Figure 2:

Molecular structure of cabazitaxel C45H57NO14



(Paller and Antonaraki, 2011)

Drugs in the cabazitaxel taxane group such as (paclitaxel, docetaxel) shows its molecular effect by destabilizing the balance of the Microtubule network (Figure 2). The drug binds to the tubular and forms a microtubule network with a more stable structure than it should be (Rathi et al.,2021) (Thus, leads to function disorders in the mitotic and interphase stages of the cell dependent on microtubules. As a result, the cell cycle cannot progress and enters metaphase which stops and triggers apoptosis of cancer cells by inducing p53 protein (Rathi et al.,2021). It has been reported that at high concentrations, the cell goes directly to necrosis (Maluf et al., 2021). Cabazitaxel, like other chemotherapy drugs, has many side effects. Most common known side effects include leukopenia, erythrocytopenia, thrombocytopenia, decrease in blood values, Gastrointestinal problems including nausea, vomiting, diarrhea and constipation, anorexia, hair loss with the formation of baldness (alopecia), bloody urine, weakness and fatigue (Rathi et al.,2021).

Irrinotecan

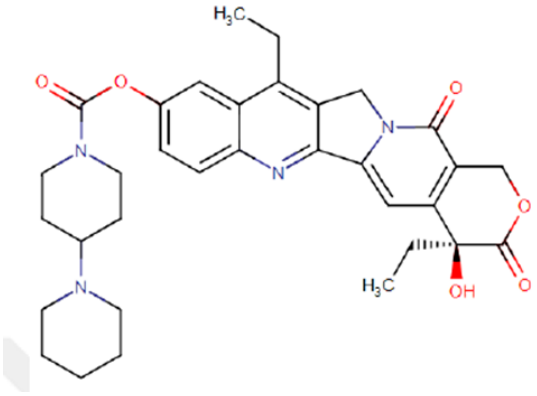
Irinotecan (IR), named Camptotheca acuminate, occurs naturally in the Chinese and Tibetan Regions. It is a water-soluble camptothecin derivative made from a tree that grows in pine trees. (Kunimoto et al.,1987). The molecular formula is C33H38N4O6 (Figure 2.8). IR is anti-neoplastic and immunosuppressive colorectal and is primarily preferred among other chemotherapy drugs in cancer treatment.

Topoisomerase I is a nuclear enzyme necessary for DNA synthesis. Topoisomerase I attaches firmly to double-stranded DNA during every stage of the

cell cycle, causing a strand separation. It stops the replication process from bending the alpha helix structure. It causes breakage of double-stranded DNA and cell death occurs (Saltz et al.,2000). Malignant cells have more topoisomerase I than normal cells and so are more sensitive to topoisomerase I inhibition (Conti et al.,1996).

Figure 3:

Molecular structure of irinotecan C33H38N4O6

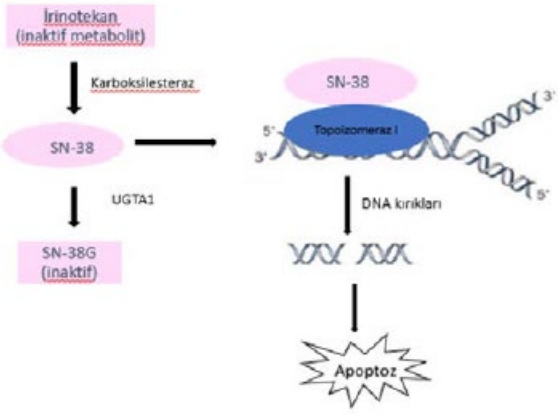


(Saltz et al., 2000)

IR is an active substance that is about a thousand times more effective than itself through carboxylesterases that is converted to metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) (Saltz et al.,2000). Secreted from the bile ducts SN38 is metabolized to inactive glucuronide (SN38G) by uridine-diphosphate glucuronosyltransferase and the excess is excreted in the urine. The enzyme that performs this process is UGT1A1. They inhibit healthy cells throught SN-38 cancer cells and UGTA1 (Figure 3). The effects of IR are diarrhoea, bone marrow suppression, skin reactions, drop in blood values, nausea,vomiting and alopecia (Saltz et al.,2000).

Figure 4:

Molecular metabolism of irinotecan



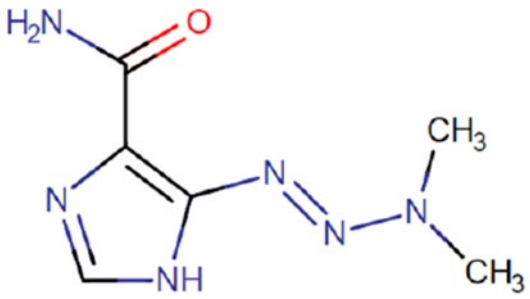
(Saltz et al.,2000)

Dacarbazine

Dacarbazine (Db) is the primary Chemotherapy drug used in the treatment of metastatic malignant melanoma.Its molecular formula is C6H10N6O (figure 5) (Robert et al.,2011).

Figure 5:

Molecular structure of dacarbazine C6H10N6O



(Robert et al.,2011)

DNA structure of 5-aminoimidazole-4-carboxamide, the precursor of purine bases. Dacarbazine is a cytostatic anti-neoplastic drug. Liver microsomal acts by converting monomethyl derivatives by oxidative N-demethylation with enzymes. The mechanism of action of dacarbazine works in two ways: First, suppresses the DNA replication, the second is synthesized and healthy by acting as an alkylating agent. It reacts with DNA structures and causes single and double strand breaks

(Robert et al.,2011). Also it activates P53 genes which causes the death of malignant cells. 24% reduction was observed when dacarbazine was used as a drug agent for the treatment of Malignant Melonoma (Robert et al.,2011). Like many chemotherapy drugs, dacarbazine acts on normal as well as cancer cell growth which leads to serious side effects. Common side effects include sterility, suppression of the immune system, emesis, vomiting, anorexia, alopecia, blood values (thrombocytopenia, leukopenia, neutropenia), anemia, increased creatinine takes (Aoki et al.,2013).

Auxiliary Drug Used in Cancer Treatment 'Leucovorin'

One of the folic acid chemically reduced derivatives is leucovorin. Folic acid acts as an antidote to drugs that act as antagonists. Leucovorin, 5-formyl derivative is a mixture of diastereoisomers of tetrahydrofolic acid (Song et al.,2018). In anti-neoplastic therapy belongs to the group of drugs called detoxifiers used. From folic acid deficiency in the treatment of megaloblastic anemia and in the palliative treatment of patients with advanced colorectal cancer. in combination with 5-fluorouracil to prolong survival. In the treatment of it is a drug used (Wong et al.,2019). Before folic acid is incorporated into DNA or protein by the body, it contains purines, pyrimidines and It is an essential B vitamin required for the synthesis of methionine. However, in this role the function to see the dihydrofolate reductase enzyme (DHFR) first with dihydrofolate (DHF) and tetrahydrofolate (THF) must be reduced to cofactors (Ter Veer et al.,2018). For the cancer treatment de novo synthesis of nucleic acids and amino acids when high-dose methotrexate is used for This important path necessary for Methotrexate, DNA in rapidly dividing cells Acts as a DHFR inhibitor to prevent synthesis of DHF and THF prevents its formation. This event is related to the deficiency of coenzymes and the result of methotrexate treatment. It causes the accumulation of toxic substances that are responsible for numerous side effects. Since Leucovorin are analogues of THF, they bypass the intracellular reduction steps of folic acid. They act as a cellular substitute for the co-factor THF, thereby preventing chemotherapeutic they prevent the toxic side effects caused by it (Wong et al.,2019).

Molecular Recognition

Biological and chemical processes are essential for a molecule to selectively recognize and bind the target molecule among molecules of similar structure. When two molecules are bound by non-covalent interactions such hydrogen bonds, electrostatic interactions, and weak metal coordination, they can only complete each other as a three-dimensional structure and exhibit molecular recognition. (Chen et al. 2002). Examples of this process are binding of the enzyme to the substrate (Tulinsky 1996), binding of the drug to a biological target (Cudic et al. 2002, Britschgi et al. 2003), antigen-antibody binding in the immune system (Jimenez et al. 2003, Sundberg and Mariuzza 2003) and The formation of mRNA from DNA templates can be given (Gitlin et al. 1988). Molecular recognition is the fundamental process that takes place in all life events.

Natural Recognition Systems

Molecular recognition in proteins

The heteropolymers of amino acids that make up proteins are lengthy, unbranched chains. Around 200–300 amino acids can be found in many proteins in the human body. The operation of every system in a biological structure depends on proteins and how they recognize other molecules to integrate these systems. Musculoskeletal movements, enzyme catalysis in digestion, taking therapeutic drugs into cells and tissues can be given as examples of events where molecular recognition of protein is essential. The best-characterized protein molecular recognition event occurs between avidin and biotin. Avidin is a tetrameric glycoprotein with four subunits, with a molecular weight of approximately 66 000 daltons. The heteropolymers of amino acids that make up proteins are lengthy, unbranched chains. Around 200–300 amino acids can be found in many proteins in the human body. The operation of every system in a biological structure depends on proteins and how they recognize other molecules to integrate these systems. (KA=1015M-1), (Pugliese et al. 1993). It is thought that lysine and tryptophan residues (especially Trp70, Trp 110, Lys 46 and Lys 94) in the avidin structure interact strongly with the carboxyl groups in the biotin structure (Gitlin et al. 1987). It is the high affinity, structural and chemical compatibility of these molecules to

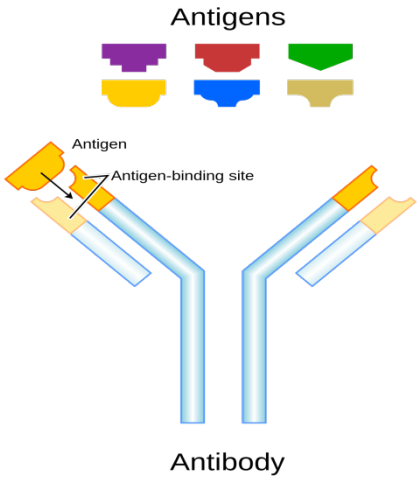
each other that makes the avidin-biotin complex the most studied interaction in recognition systems based on non-covalent interactions.

Molecular recognition in the immune system

Another event where molecular recognition takes place is the antigen-antibody interaction that takes place in the immune system. Antibodies are globular proteins containing specific binding sites that can recognize and bind foreign substances in the bloodstream. Foreign substances called antigens can be a simple protein or a large bacterium or virus.

Figure 6:

Recognition between antibody and antigen

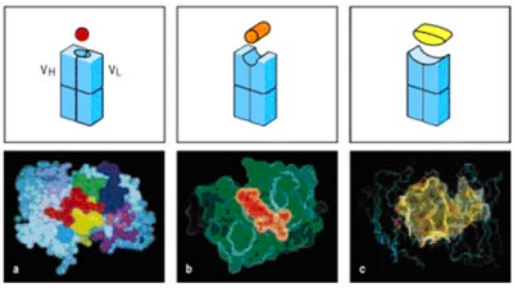


(Rachkov and Minoura 2001)

The "Y" structure of antibodies is made up of two identical polypeptide chains, the heavy chain (55 kDa), and the light chain (25 kDa), which are connected by disulfide bonds to form linear amino acid helices. The epitope, a changeable amino acid sequence that can detect a specific area of antigens, is located at the N-terminal end of the chains. The orientation of the binding area created by the heavy and light chains, in addition to the amino acid sequence, gives recognition. (Figure 6).

Figure 7:

Binding of antigens to appropriate (a) gaps (b) slits or (c) surfaces in the binding site of antibodies



(Bergman, 2005)

Electrostatic forces, hydrogen bonds, Van der Waals forces, hydrophobic forces, or a combination of all these non-covalent interactions contact the epitope of an antigen with the variable region of an antibody. (Haupt and Mosbach, 1998).

For the immune system to function, antibodies have to recognize a large number of different molecules. Thanks to the diversity and flexibility in amino acid sequences, a large number of possible chemical target molecules can be recognized. Some researchers estimate that the human immune system has between 107 and 109 different antibody structures. Many researchers are making efforts to prepare artificial antibodies for the treatment of diseases by taking epitope binding as an example (Rachkov and Minoura 2001) (Vlatakis et al., 1993) use these artificial antibodies as enzyme inhibitors to create libraries for potential new drugs and to identify target molecules for protein binding.

Molecular recognition in synthetic polymeric matrices

Researchers have sought to create totally synthetic polymer materials that specifically bind to the target molecule in light of the developments in protein chemistry and the understanding of protein recognition. Such materials have a wide range of possible applications, including controlled medication release, chromatographic column filler, and solid phase extraction. Today, most of these polymers are prepared in the presence of the target molecule. The purpose of this is to ensure that the chemical functionality and three-dimensional structure of the

target molecule are memorized in the synthetic matrix so that the target molecule can be recognized and thus biological recognition can be imitated. In synthetic structures, it is possible to add many different functional properties to the structure and to provide mechanical stability.

Molecular imprinting

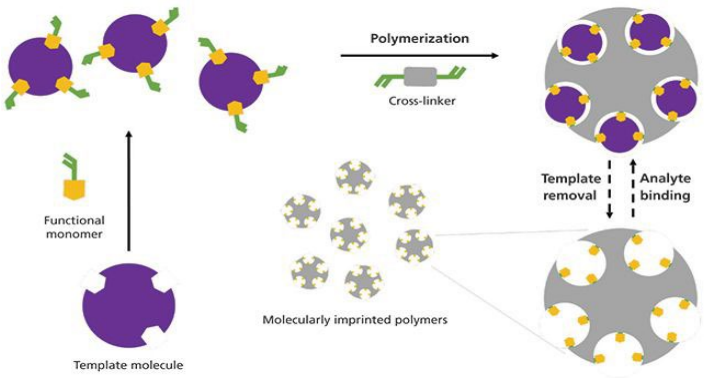
The idea of molecular imprinting was inspired by Nobel laureate Linus Pauling's theories on antibody formation. (Ansell et al. 1996, Mosbach and Ramstrom 1996). Pauling hypothesized that antibodies behave like denatured proteins, do not contain hydrogen bonds, and their chains can move freely. When it encounters the antigen, the event called "molecular complementation" takes place and the functional groups in the chemical structure of the antigen interact with the amino acids in the antibody. This way, the antibody memorizes the structure of the antigen (Pauling 1940). Later, this hypothesis was proven wrong, but the idea that freely moving polymer chains could be organized in a complementary way around a molecule inspired the phenomenon of molecular imprinting.

Based on the molecular recognition event in many biological events such as immune response, ligand-receptor interaction and enzyme catalysis, researchers use this specific recognition event in nature to prepare synthetic receptors in order to conduct better, more selective and sensitive analytical studies. gave rise to a new known field.

Molecular imprinting is based on the fact that functional monomers are organized around the template molecule and polymerization is carried out with the addition of crosslinkers, and a new material containing binding sites specific to the template molecule in shape and size is obtained (Figure 8) .

Figure 8:

Schematic representation of the molecular imprinting method



(Tamayo et al. 2007),

The molecular imprinting method basically consists of three steps:

(1) Pre-complexation

(2) Polymerization

(3) Removal of template (target) molecule

In the pre-complexation stage, the template molecule (a small molecule, a biological macromolecule, or a microorganism) is a polymerizable monomer containing complementary functional groups and a reversible covalent bond(s), electrostatic interactions, hydrogen bonds, Van der Waals interactions, hydrophobic interactions, or a It interacts with the metal by forming a coordination bond. A crosslinker, an initiator and a specific analyte are added to the prepolymerization mixture (pattern molecule-monomer complex). A porogen is added to form the pores that will allow it to reach the binding sites easily.

In the polymerization stage, the monomer template complex is polymerized thermally or photochemically at low temperature, depending on the characteristics of the template molecule used. Due to the formation of chemical bonds between the monomer and crosslinker molecules, the position of the functional monomers around the template molecule is fixed when polymerization is performed. In the last step, with the removal of the template molecule, a three-dimensional polymeric structure is prepared, containing specific binding sites for the template molecule in terms of shape, size and functional group. In other words, the steric and chemical information of the imprinted molecule is transferred to the polymer structure.

This technique is simple and inexpensive, and the obtained molecularly imprinted polymers are highly selective, have excellent mechanical strength, and are resistant to temperature, acidic and basic conditions, and organic solvents. This method includes solid phase extraction (Tamayo et al. 2007), liquid chromatography (Sellergren 2001), drug release systems (Cunliffe et al. 2005), capillary electrophoresis and electrochromatography (Turiel and Martin-Esteban 2005), enzyme-like catalysis (Hall va. et al. 2005) or sensor development (Ye and Haupt 2004a).

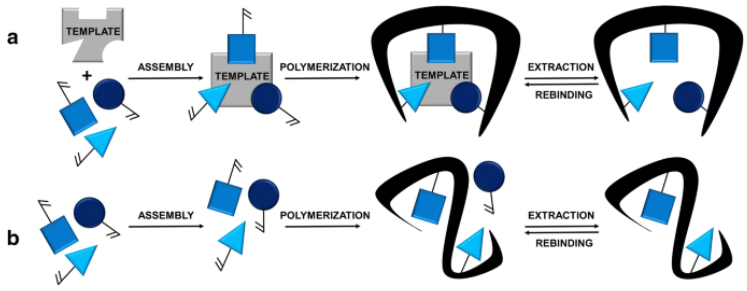
Approaches used in the preparation of molecularly imprinted polymers

Two different approaches developed in molecular imprinting to date are covalent and non-covalent imprinting (Figure 9). The covalent approach was described by Günter Wulff et al. in 1972 (Wulff and Sahran 1972). In covalent imprinting, the template molecule is covalently attached to a polymerizable functional monomer (boronate ester, ketal and acetal or schiff bases). After copolymerization using a crosslinker, the template molecule is covalently bonded through the highly crosslinked polymer.

It is removed by crushing and molecular cavities are obtained that specifically recognize the template molecule.

Figure 9:

(a) Non-covalent (b) Schematic representation of the covalent molecular imprinting method



(Hillberg et al. 2005)

On the other hand, non-covalent suppression is an approach carried out by Klaus Mosbach et al. in 1981 (Andresson et al. 1984). In this approach, specific binding sites are formed by pre-organization and subsequent cross-linking between

template molecule and functional monomer. The template molecule is bound to the binding sites by non-covalent interactions such as hydrogen bonds, hydrophobic interactions, van der Waals interactions, and Coulomb interactions between ionic groups during both pre-organization and back-bonding.

Non-covalent imprinting is a more commonly used approach in the preparation of molecularly imprinted polymers due to the simplicity of the method and has many advantages over covalent imprinting. The most important advantages are that the pre-complex can be prepared by simply mixing the template molecule and the functional monomer, and the template molecule can be easily removed from the structure because the interaction is not covalent. Non-covalent imprinting is a much more promising approach because many molecules, including biological molecules, have the capacity to engage in non-covalent interactions with functional monomers.

The major limitation of non-covalent molecular imprinting is the binding conditions specific to the molecule under study. In most cases, the interaction between the monomer and the template molecule is hydrophobic and the polar environment disrupts these interactions. Another limitation is the need for more than one region for interaction. Molecules with a single interaction site, eg only one carbonyl group, often result in molecularly imprinted polymers with limited molecular recognition ability.

Molecular recognition event depends on many factors such as three-dimensional structure and functional compatibility. Considering the functional complementarity, it is the template molecule, monomers and the polymerization reaction that determines the quality and performance of the formed molecularly imprinted product, even if all non-covalent interactions occur for the molecular recognition that will take place between the template molecule and the molecular binding site. Moreover, the number and quality of molecular recognition sites are closely related to the properties and formation mechanism of the pre-complex formed between the monomer and the template.

(1) template molecule, (2) monomer, (3) crosslinker, (4) solvent, (5) initiator and initiation method, and (6) polymerization method itself used in the preparation of molecularly imprinted polymers are used to determine the chemical, morphological

and molecular recognition properties of the prepared polymer. (Yan and Row 2006, Cormack and Elorza 2004).

(1) Template molecule

It has a central importance in all molecular imprinting methods, as the template molecule interacts with the functional monomer and affects the number and quality of molecular recognition sites. For many reasons, it is not possible to use all molecules as templates. Since molecularly imprinted polymers are mostly synthesized by free radical polymerization, the template molecules must be chemically inert under polymerization conditions for polymerization to take place. If the template molecule participates in radical reactions and is not stable under polymerization conditions, alternative imprinting strategies should be determined. The properties sought in a template molecule can be listed as follows:

(a) The template molecule must not contain polymerizable groups.

(b) The template molecule should not contain groups or residues (such as thiol, hydroquinone residues) that could prevent or slow down free radical polymerization.

(c) The template molecule must be resistant to UV light or temperatures at which polymerization will take place.

Drugs, amino acids, carbohydrates, proteins, nucleotide bases, hormones, pesticides, coenzymes and ions can be used as molecules to be imprinted. When an ion is used as the imprinted molecule, the selectivity of the polymeric material depends on the charge, size, coordination number and geometry of the imprinted ion.

(2) Functional Monomer

Functional monomers are in charge of the binding interaction at the imprinted binding site, and typically, in the non-covalent imprinting method, more monomer is used than template molecule in order to shift the reaction in the direction of complex formation during the formation of the pre-complex between

the two. The functionality of the template molecule and the functionality of the monomer must be compatible in order to improve the complex formation and suppressive impact.For example, if one is an H-bond donor, the other must be an H-bond acceptor. However, if the polymerization is to be carried out by using two or more monomers at the same time, the ratio of the reactivity of the monomers should be appropriate. The pre-complexation that will take place between the template molecule and the monomer will affect the electronic and steric structure of the monomer and change the reactivity of the monomer. Functional monomers of different chemical structure and polarity are commercially available or can be synthesized. Figure 9 shows the structures of some important functional monomers used in non-covalent imprinting.

(3) Crosslinker

Determining the crosslinking polymer matrix's shape (gel type, macroporous, or microgel powder) in an imprinted polymer, assuring the stability of the binding site, and giving the polymer matrix mechanical strength.It undertakes three main tasks. Although there are many studies on the effect of the amount of crosslinker used on the molecular recognition capacity, when the issue is considered from the point of view of polymerization, high crosslinking rates are generally used to obtain macroporous materials with sufficient mechanical strength, and the crosslinking rates in the prepared polymers exceed 80%. The high cross-linking prevents the polymer from dissolving, making it easy to use.

The crosslinker's and the functional monomer's reactivity during polymerization must be appropriate. If the functional monomer or crosslinker is excessive, copolymerization will not occur effectively. Another crucial factor is the crosslinking agent to functional monomer ratio. If the mole ratios are too tiny, the target molecule's binding sites are blocked by the template molecules' binding sites because they are too close to one another, and an effective outcome cannot be attained. In very large molar ratios, the effectiveness of the imprinting is also reduced as a result of non-covalent interactions of the crosslinker with the functional monomers or the template molecule.

There are many crosslinkers commonly used for molecular imprinting. Most of these are produced commercially, and very few of them act as functional

monomers by interacting with the template molecule. Ethylene glycol dimethacrylate (EGDMA) and divinyl benzene (DVB) are widely used crosslinkers for molecular imprinting in organic solvents, and N,N' methylene bisacrylamide in water.

(4) Solvent

The solvent used in molecular imprinting is the component that ensures that all the components used (pattern molecule, functional monomer, crosslinker and initiator) are in a single phase. Another important function is the formation of pores in the macroporous polymer. For this reason, the solvent is often referred to as a porogen.

The properties and amount of the pore former used in the preparation of macroporous polymers are the most important factors that determine the morphology of the polymer and the total pore volume. When a thermodynamically suitable pore former is used, the resulting polymers generally have both a good pore structure and a high specific surface area. Increasing the amount of pore builder used also increases the pore volume.

The solvent's additional function during the polymerization procedure is to distribute heat. Otherwise, the reaction mixture's temperature will be too high locally and unwanted side reactions may happen. The choice of solvent also depends on the type of printing. If a solvent is able to completely dissolve all of the components, it can be employed in covalent imprinting. On the other hand, the solvent employed in non-covalent imprinting should have the ability to increase the interaction between the template molecule and the functional monomer as well as the imprinting effect. While apolar, aprotic solvents such as toluene stabilize the formation of hydrogen bonds, water is a good option if the hydrophobic interaction is to be used for complexation.

(5) Launcher

In principle, all initiation methods used to initiate free radical polymerization can also be used in the presence of the template molecule. However, the choice of initiator will be different for the molecule used as the template. If the template molecule is photochemically or thermally unstable, consideration should

be given to selecting the appropriate initiator. In addition, lower polymerization temperatures will be preferred if hydrogen bonds are active in the pre-complex formation, and in this case, photochemically active initiators that can be used at low temperatures should be preferred.

The high selectivity recognition of the template molecule and its binding by imprinted polymers depend on the physical and chemical properties of the material (such as flexibility, number of binding sites, and structure of the material).

In addition to selectivity, desorption and backlinking kinetics must be quick under favorable conditions for molecularly imprinted polymers to be more useful. Therefore, while creating molecularly imprinted materials, it is crucial to choose the right binding interactions. The presence of more than one binding site ensures that the interactions between the binding sites of the monomer and the template molecule are better, thus making molecular recognition more selective. The diversity of molecular interactions affects the degree of selectivity and reversibility. For example, interactions with covalent bonds are highly specific, but backlink kinetics are slow. However, the kinetics of hydrophobic interactions are faster, but their selectivity decreases. In general, non-covalent interactions have wider applications due to their applicability to many compounds, fast kinetics, and bond formation and breaking properties under more favorable conditions. Moreover, certain non-covalent interactions such as π-π interactions, hydrogen bonds and hydrophobic interactions hold promise for the design of new molecularly imprinted functional polymers.

Biosensors

Thanks to the developments in life sciences (genomics, proteomics, molecular engineering), new methods have been developed for the treatment of many diseases, public health has been brought to a higher level and life expectancy has increased. In developed countries, diseases such as cardiovascular diseases caused by lifestyle have become the main public health problem and have become one of the most important causes of death. For this reason, studies on finding biological markers that will allow the diagnosis of diseases in the field of health and even before their symptoms appear, and developing detection methods for them have gained momentum. In addition, monitoring the change in the amount of these

substances in body fluids also allows the development of the disease to be monitored. Biomarker proteins have been identified for some diseases and are used clinically. However, there are still attempts to find reliable biomarkers for some diseases. Today, different techniques are used in hospitals and private laboratories for the determination of biomarkers in body fluids. Traditional immunoassay-based methods include radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), immunoagglutination assay (IAA), and fluorescence immunoassay (FIA). These tests include benefits including high sensitivity, low detection limits, and a wide detection range. Antibodies are used as the biological recognition element in immunoassays and are one of the fastest growing detection methods due to advances in antibody engineering. However, these methods are very tedious and time consuming. Also, the chances of linking automation and operational phases are limited (McGlennen 2001). Labeling and additional substances to show the binding between the analyte and the receptor are generally needed for recognition (Peter et al. 2001). Labeling prolongs the test time, increases the cost, and may disrupt the receptor binding site, resulting in false-negative results. Furthermore, fluorescent compounds are mostly hydrophobic and noise is a problem in many methods, causing false positive results.

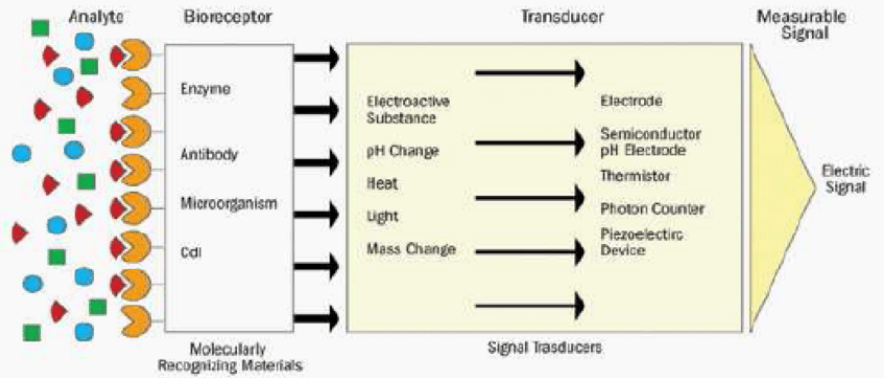
An ideal imaging platform should be fast, precise, specific, reliable and easy to use. In addition, samples such as blood, urine, plasma, saliva, cerebrospinal fluid for analyte determination; It should be able to be used directly without the need for a sample preparation step. The devices used should be capable of allowing continuous monitoring of the analyte concentration, which is not possible in conventional homogeneous or heterogeneous immunoassays.

Biosensors can be a suitable alternative to current diagnostic methods and have the potential to meet the requirements. A biosensor is an analytical instrument that uses the high selectivity of biomolecules as a molecular recognition tool and detects the target analyte from a complex mixture. In general, a biosensor consists of a molecular recognition layer consisting of biomolecules that selectively bind to the target molecule to be analyzed in the sample, and a converter system that converts the biochemical event that takes place there into a quantitative electrical signal (Figure 10). Catalytic biosensors and affinity biosensors are two different types of biosensors that are utilized for molecule recognition. (Ramanavieius et al.,

2005). The recognition component (enzyme, cell, or tissue) in catalytic biosensors changes the substrate molecules into products, enabling signal amplification. The recognition molecule (antibody, nucleic acid, peptide, cell receptor, and protein) binds to the analyte in affinity biosensors. Together with transducers including electrodes, transistors, thermistors, and optical instruments, these two types of biosensors are used.

Figure 10:

Structure and components of a biosensor



(Ramanavieius et al. 2005).

Electrochemical sensors, a type of catalytic biosensor, are biosensors used to determine the levels of substances such as insulin, glucose, hCG, theophylline, α1-glycoprotein, apolipoprotein E, FSH, LH in the blood or urine (Morgan et al. 1996). These electrochemical sensors have advantages such as simplicity and high sensitivity. Electrochemical sensors developed to date have disadvantages such as low selectivity in the presence of interfering substances in body fluids. However, the most successful type of biosensor developed to date is enzyme-based amperometric sensors, commercially available for glucose, lactate, urea, etc. They are used as disposable sensors for the determination of substances.

Molecular Recognition Layer

Antibodies

The produced sensors are known as immunosensors, and antibodies or antigens are typically used in biosensors as a molecular recognition tool. The purpose of this part is to combine the complex generated between the antigen and

the antibody with the sensor to increase its sensitivity and selectivity. Immunosensors use monoclonal or polyclonal antibodies. Particularly in competitive immunoassays, polyclonal antibodies are still frequently utilized as primary antibodies. Its primary benefit is the relatively straightforward preparation process, which is also inexpensive. Two-antibody sandwich immunoassays can be prepared with a single type of polyclonal antibody and are much simpler than systems using two types of monoclonal antibodies or monoclonal-polyclonal antibody mixtures. With the immunization of a large animal, a long-term supply is made possible and they are typically specific for their intended use. However, polyclonal antibodies are a heterogeneous mixture of antibodies derived from many different B-lymphocytes in terms of binding affinity, isotype, and specificity. As a result, antibodies obtained from each animal at each different expedition have their own characteristics. In this case, a proper signal/concentration ratio is achieved only with antibodies obtained from a single animal at a time. Antibodies obtained at different times will cause the signal ratio to vary in immunoassays. This creates difficulties in determining the amount of the antibody to be used in order to design a uniform assay in an immunoassay. Polyclonal antibodies also detect epitopes in the immunogen and the contaminants injected with it, which is another benefit. This results in non-specific binding, raising the immunosensor's detection threshold. Although polyclonal antibodies obtained from animal serums were used in the first immunoassays, today the immunoassay industry prefers monoclonal antibodies produced by fusion technology. Only one kind of antibody can be produced by hybridoma cells (monoclonal antibodies). It is necessary to vaccinate again every time new antibodies, whether monoclonal or polyclonal, are produced. This process is a long and tiring process that does not always guarantee success.

Alternative recognition elements used in immunosensors

Although polyclonal or monoclonal antibodies are widely used in immunoassays, the use of these proteins has some disadvantages for immunoreactions. These are as follows: (1) The structure of the molecule may alter and become unsuitable for immunoreactions if the working conditions differ from body characteristics or conditions. (2) During the regeneration process, the immobilized antibody may be damaged or detached from the surface. Only acquiring antibodies with high affinities (>1010 M-1) will result in adequate

analytical sensitivity (Hock 1997). As a result, the usage of immunosensors in disposable devices is constrained due to a high affinity constant and a labile immobilized antibody that limit surface renewal in practical applications (Morgan et al. 1996). (3) The analysis time is increased because the transducer's detection time is less than the reaction period between the antibody and the antigen. As the molecular recognition region of the biosensor, novel ways have been developed to address these issues. These include the following:

(1) Aptamers

Aptamers are artificial single-stranded DNA or RNA oligonucleotide pieces that have a high affinity and specificity for recognizing target molecules like nucleotides, medications, and proteins (Luppa et al. 2001). When it comes to adhering to the immunosensor's surface, aptamers have superior qualities to antibodies, and they may be produced repeatedly and in desirable quantities using synthetic methods. Their high cost and instability are the problems that need to be solved before these molecules can be used in immunoaffinity biosensors.

(2) Antikalin

Lipocalin can be used to create anticlines by random mutagenesis of different amino acids, which is a promising method that can be utilized as an alternative to recombinant antibody fragments. Lipocalins, a family of proteins involved in the storage and transport of hydrophobic and/or chemically sensitive organic substances, are similar to retinol-binding proteins. These binding proteins share a common -barrel shape that is coiled around the central core by eight antiparallel -sheets. The bilirubin-binding protein, for instance, can be selectively altered to complex with potential antigens like digoxigenin (Schlehuber et al., 2000). The synthesis and stability of anticlins, as well as the magnitude of the affinity constant, remain unresolved issues.

(3) Molecularly imprinted polymer (MIP)

Biological receptors have specific molecular affinity and although they are widely used in diagnostic sensors, they are produced with high cost complex protocols, require favorable conditions due to their low stability, and there are no natural receptors for many analytes. (Whitcombe et al. 2000, Wulff 2002, Haupt et

al. 2000, Ye and Haupt 2004a). Therefore, there is a need to synthesize artificial recognition elements.

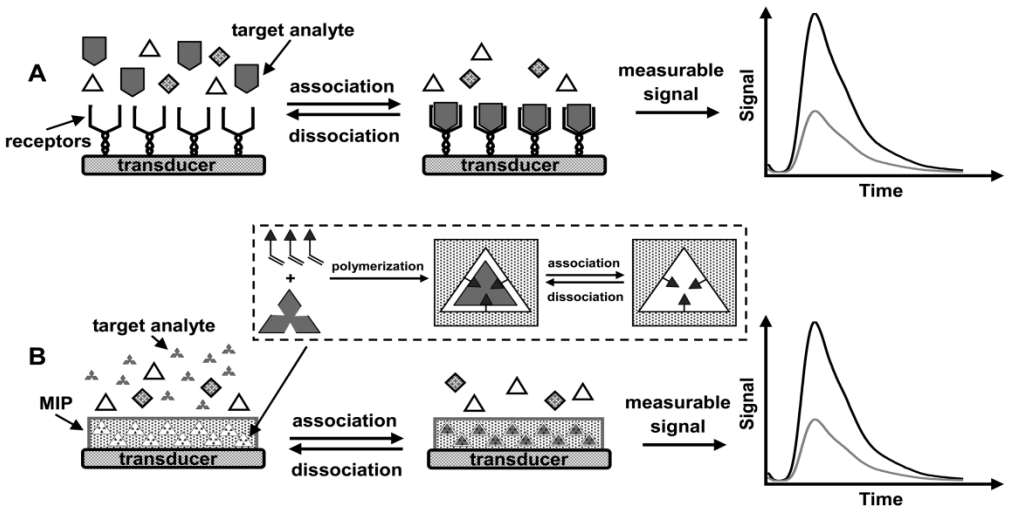
Molecular imprinting is one of the most effective methods that can be used in the preparation of artificial recognition elements (Guan et al. 2008). This synthetic technique is simple and inexpensive, and the resulting molecularly imprinted polymer has high selectivity, excellent mechanical strength, resistance to temperature, acidic and basic conditions, treatment with organic solvents, and better engineering properties compared to biological receptors (Wulff 2002, Haupt and Mosbach 2000). , Ye and Haupt 2004a). Furthermore, this approach provides the opportunity to develop sensors for analytes that do not have natural receptors.

Due to these characteristics, MIP materials can be widely used as recognition elements in many fields such as life, pharmaceutical and environmental sciences (Andersson et al. 1990, Ramstrom et al. 1996, Spivak 2005). Although its main use is in selective separation, the detection of active molecules, the development of MIP-based sensors for pharmaceuticals and pollutants is one of the most ambitious subjects and has attracted considerable attention in recent years (Piletsky and Turner 2002, Holthoff and Bright 2007, Hillberg et al. 2005.

In biosensors, the signal is generated by binding the analyte to the recognition element in the molecular recognition layer. Then the transducer converts this signal to a measurable size and provides a resultant data. The same principle can be used when using molecularly imprinted polymers instead of biomolecules as a recognition element (Figure 11).

Figure 11:

(A) Antibody-based sensor (B) Working principle of MIP-based biomimetic sensor



(Stephenson and Shimizu 2007).

The analyte's general qualities or modifications to the system's physicochemical characteristics brought on by the analyte's binding are employed as the basis for detection. This idea is broadly applicable and essentially unaffected by the analyte's composition. As an alternative, certain compounds can be utilized to improve sensor response or signaling. When an analyte has particular characteristics, it may be possible to prepare MIP-based sensors using those characteristics.

Transducers used in biosensors

According to how they generate signals, the transducers used in biosensors can be categorized into four main groups: thermal transducers, optical transducers, transducers sensitive to mass change (piezoelectric, acoustic wave), and electrochemical transducers (amperometric, potentiometric, conductometric, and capacitive) (calorimetric).

Depending on how a biosensor detects, it can be classified into two categories: (1) direct detection and (2) indirect detection with or without labeling for antigen-antibody interaction. Different physical properties, including refractive index (SPR transducer), mass change (QCM transducer), and dielectric constant, change as a result of coupling in the direct sensor (electrochemical transducer). A signal-generating label on one of the molecules in the immune complex is

necessary for indirect sensing. A separate step is needed for this tag to create a change in the feature. These sensors have very different labels that are generally used in immunoassays (Morgan et al. 1996, Luppa et al. 2001, D'Orazio 2003). Direct sensors shorten analysis time and allow simultaneous determination of bound analyte. These sensors can be produced even more cheaply when labels are not used. Thanks to the advances in recent years, the preparation of direct sensors has developed and recently these sensors have become the preferred immunosensors in medical applications.

The most often used immunosensors for bioanalysis at the moment are optical ones because of their advantages like quick signal production and reading. The most often used optical immunosensor for tracking immunoreactions in clinical chemistry is a direct optical transducer, such as the SPR transducer (Luppa et al. 2001).

The main advantage of the direct optical transducer is that no marking is needed for detection, thus eliminating the separation step required to separate the bound species from the free species. Moreover, the magnetic field component of the light only affects the region very close to the surface, thus preventing interference with other substances in the sample (D'Orazio 2003).

High sensitivity QCM is used as a transducer in the sensor. SPR and QCM are both based on wave-scattering phenomena and exhibit a resonance structure, despite the fact that QCM detects the change in mass rather than an optical characteristic. A common type of direct immunosensor is QCM. While the QCM approach analyzes the frequency variations linked to the change in the amount of mass bound to the surface, the SPR technique examines the refractive index change in the region immediately next to the surface.

When SPR biosensors are compared with QCM sensors in terms of protein adsorption or antigen antibody recognition; they have very close characteristics in terms of sensitivity, monoclonal antibody and serum determination limits (Koesslinger et al. 1995). Although the QCM device is cheaper and easier to use, SPR sensors have many advantages compared to QCM. SPR sensors have a shorter response time and the SPR technique is cheaper (Laricchia-Robbio and Revoltella 2004). Moreover, the recognition area in the SPR system is smaller than in QCM

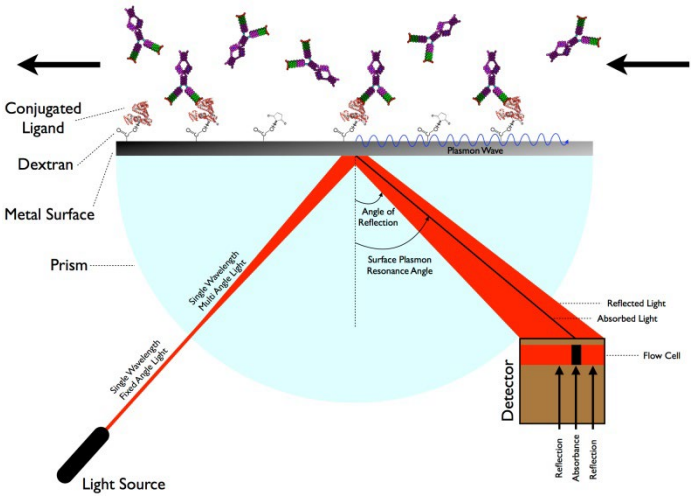
sensors. As a result, smaller flow cells can operate with smaller sample volumes than QCM sensors since fewer molecules are needed to produce the same surface density. In order to create a complete analysis system known as "lab on a chip" that simultaneously incorporates sample preparation, chemical analysis, and data interpretation elements, SPR transducers can thus be more readily combined with microfluidics.

Surface Plasmon Resonance (SPR)

When polarized light is sent to the sensor surface, the surface of which is covered with a thin metal film, the light will be reflected from this surface, which acts like a mirror. When the intensity of the reflected light is monitored by changing the angle of incidence, it will be seen that the intensity of reflection of the light passes through a minimum (Figure 12)

Figure 12:

Stimulation of surface plasmons



(Laricchia-Robbio and Revoltella, 2004)

At a certain angle of incidence, the light excites the plasmons on the metal surface and surface plasmon resonance occurs. Photons of p-polarized light interact with free electrons on the metal surface and cause wave-like vibration (resonance) of free electrons, resulting in a reduction in the intensity of the reflected light. The angle at which the maximum loss to the intensity of the reflected light occurs is

called the resonance angle or SPR angle. The SPR angle depends on the optical characteristics of the system, namely the refractive indices on both sides of the metal (usually gold). While the index of refraction on the prism side is unchanged, the index of refraction in the immediate vicinity of the metal surface will change due to the mass collected on it (eg protein). When the refractive index changes, there will be a shift in the angle at which the reflected light intensity decreases to a minimum, as shown in Figure11. (A) shows the reflected light intensity against the angle of incidence, while (B) shows after the change in the refractive index. The surface plasmon resonance event can show not only the difference between these two steps, but also the change over time if the shift in the resonance angle at which the minimum is observed is observed.

Figure 12 shows the shifts in the SPR angle and is called the sensogram. If this change is due to a biomolecular interaction, the kinetics of the interaction can be studied simultaneously. SPR sensors can detect a very limited area on the metal surface or a fixed volume. The depth of penetration of the electromagnetic field (the vanishing field) from which the signal can be received usually does not exceed a few hundred nanometers and decreases exponentially with distance from the metal on the sensor surface. The depth of penetration of the lost field is a function of the wavelength of the incident light.

SPR sensors do not have an intrinsic selectivity. All refractive index changes are reflected as signal changes. These changes may be due to the different refractive indices of the media. For example, a change in the buffer composition or concentration, as well as the adsorption of different materials on the sensor surface, can cause a change in the index of refraction. The amount of adsorbed species can be determined after injection of the equilibrium buffer. For selective recognition on the SPR sensor surface, the surface of the sensor must be modified with a ligand that can selectively capture the target molecule but not affinity for any other component in the sample or buffer composition.

Surface plasmon resonance system

SPR devices consist of three basic parts integrated within a system: optics, liquid system and sensor chip. Devices differ in terms of optics, fluid systems and degree of automation, which affects their performance. In addition, the quality and

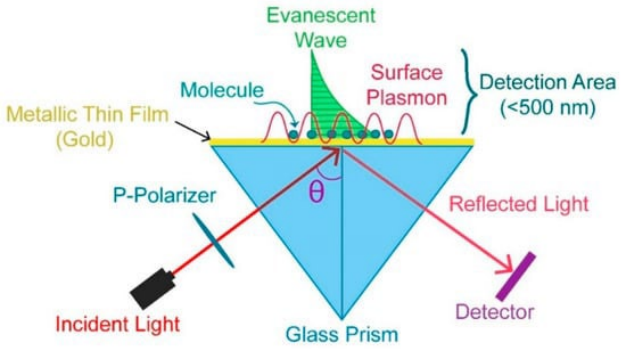
properties of the sensor chips affect the measurements of the interactions of biomolecules.

SPR devices are configured in different ways to determine the shift in SPR angle. Three different optical systems are generally used to excite surface plasmons: prisms, gratings and optical waves. However, the prisms prepared in the Kretschmann configuration are the most widely used in devices.

In this configuration, a prism couples the p-polarized light to the sensor surface coated with thin gold metal, as shown in Figure 13. The light is reflected to the detector, which will measure its intensity using a photodiode or a camera.

Figure 13 :

Kretschmann configuration used to stimulate surface plasmons



(Kretschmann and Raether, 1968)

In devices with grating couplers, the light is reflected onto the substrate with a lower refractive index. In practice, this means that light travels through the liquid before photons generate surface plasmon waves, as in ellipsometric devices. In addition, some devices use optical waves to measure drift. The phenomenon common to all configurations; to measure the refractive index changes on the sensor surface, to take direct, unmarked and simultaneous measurements.

SPR sensors are capable of measuring very low amounts of chemical and biological compounds near the sensor surface. Detection of the biomolecular binding event occurs by collecting biomolecules on the sensor surface. Because in this case, the biomolecules replace the electrolyte used as the balancing solution,

causing a change in the refractive index. Protein molecules have a higher refractive index than water molecules (Δn ~ 10 -1). The sensitivity of most SPR devices is in the range of Δn ~ 10 -5 or 1pg protein/mm2.

Use of surface plasmon resonance technique for clinical diagnosis

In recent years, the use of surface plasmon resonance-based detection methods in clinical applications has attracted attention. With the studies, SPR-based sensors have been developed for biomarkers, hormones and drugs of many diseases. Most of these assay experiments were performed on pure samples with minimal or no matrix effects, but were not studied with clinical samples.

Companies that manufacture SPR systems make up a wide range (such as Biacore, IBIS, GWC Technologies, GenOptics, Biosensing Instruments, K-Mac and Lumera). However, these devices are intended for use in the research field. Today, there are no portable SPR detection platforms that facilitate clinical recognition, can perform multiple measurements in human samples at the same time, and require a small number of trained people. Detection by SPR in clinical samples is very convenient for several reasons.

1. Detection is fast. Since the detection is based on the refractive index change in the near-surface region, the specific binding event can be monitored as soon as it occurs. Time-consuming washes are not required to remove unbound reagent. This shortens the time considerably compared to methods such as fluorescence.

2. There is no need to mark the target molecule for detection. Labeling the molecule can affect its binding kinetics and affinity. It also increases the complexity of the method and the cost of reagents.

3. SPR-based immunoassays are sufficient to be used in the determination of many substances with clinical use. In cases where the sensitivity or detection limit is insufficient, methods that provide signal amplification can be used to adjust these two parameters in the best way.

4. SPR requires a simple optical system that can be miniaturized enough to be used efficiently in clinical applications.

5. The SPR system can simultaneously detect multiple binding events at individually defined sites. The number of simultaneous measurements depends on the separation capability of the device and the density of the components on the captured platform.

6. Non-specific binding of interfering substances present in complex samples to the SPR surface can be reduced by using multiple reference surfaces.

7. Recent advances in microfluidics allow preconditioning the flow of low volume samples at the SPR surface. In this way, it is possible to minimize the contamination of the surface with non-specific bonding. In addition, preconditioning complex samples can significantly reduce expected detection intervals, thus further simplified sample analysis.

The basic requirements for the development of an SPR-based recognition system with clinical use can be listed as follows:

(1) Mechanical and optical simplicity for miniaturization and low cost

(2) An adequate performance to detect the substance to be analyzed.

(3) Ability to be used durably under changes in temperature and refractive index

(4) Developing bioassays that can make rapid and quantitative analyte measurements simultaneously from complex samples

Molecular Imprinted Polymers and Optical Sensor Applications

Molecular imprinting is an event that occurs with the formation of specific recognition sites for the template molecule. Synthetic receptors prepared using this technique are very attractive materials due to their high affinity, specificity, low cost and durability compared to natural receptors, enzymes and antibodies used in biosensors.

Compared to their biological counterparts, the use of molecularly imprinted polymers as recognition elements in sensors has some advantages: (Kandimalla and Ju 2004)

In principle, molecularly imprinted polymers can be prepared for any compound, and animals do not need to be used for production, as with antibodies.

Because of their highly cross-linked nature, molecularly imprinted polymers are more stable than antibodies. Therefore, they can be easily used in acidic or basic conditions, in the presence of organic solvents or at high temperatures and pressures.

Molecularly imprinted polymers can be prepared inexpensively and easily and can be stored in a dry environment at room temperature without losing their recognition capacity.

Generally, MIP production is fast and inexpensive. In addition, the prepared material can be used over and over again.

The synthesis of these polymers is fully compatible with lab-on-a-chip and nanotechnology.

Recently, intensive efforts have been made to prepare protein imprinted polymers. The low solubility of proteins in organic solvents, the deterioration of their three-dimensional structure during dissolution, and some other problems limit the preparation of protein-selective molecularly imprinted polymers. However, the preparation of protein-imprinted polymers is an interesting and challenging field not only in sensor preparation, but also in medicine, diagnosis, proteomics, environmental analysis, and drug release.

The use of molecularly imprinted polymers as recognition elements in optical sensors also has some disadvantages:

Difficulties in converting the integration and coupling event with the transducer into a measurable optical signal. To overcome these limitations, new monomers with different functionalities or labeled template molecules are developed and used to obtain the sensor response.

Molecularly imprinted polymers usually have heterogeneous binding sites, unlike biological receptors that have selective and distinct binding sites, such as monoclonal antibodies.

In most cases, compared with biological receptors, their affinity constants are lower and their binding kinetics slower than with biological receptors.

Selective recognition is limited in aqueous solution, where biomolecules can perform excellently.

MIP synthesis usually requires the use of large amounts of template molecules. This can cause a limitation if an expensive or toxic template molecule is used. However, in principle, it is possible to recover these molecules after polymerization or to use synthetic analogs of the molds.

The use of these materials in optical sensor preparation is slowly increasing in parallel with the availability of new monomers and labeled analyte derivatives with suitable functionality and the optimization of polymer preparation procedures that allow efficient integration with the transducer.

Because they are synthetic materials, it is a natural result that imprinted polymers are more stable than enzymes or receptors. The main reason behind this high degree of stability is the high rate of crosslinking, which ensures that the bonding sites formed in the polymer prepared by imprinting are rigid. Imprinted polymers are resistant to acidic and basic conditions and to treatment with organic solvents. They are also stable at high and low pressures or extreme temperatures.

It is a relatively inexpensive process for the preparation of artificial receptors by imprinting polymerization. In most cases, the cost of an imprinting procedure depends only on the template molecule used. Moreover, if the template molecule is expensive, it is possible to recover and reuse the template molecule. Alternatively, inexpensive template molecule analogs can be used for MIP preparation. In general, we can say that; The cost of MIP preparation is 3-4 times cheaper than the production of natural receptors, which makes MIP technology competitive with other methods.

The use of molecularly imprinted polymers with organic solvents opens up new application areas such as biomimetic sensors and use in chemical and pharmaceutical production. Areas such as quality control and online monitoring of the production process are quite interesting.

One of the most important problems with multisensor preparation is the significant differences in the performance of natural receptors and enzymes. Each of these molecules has different stability, activity and sensitivity, and in most cases requires different substrates and different buffer systems with different ionic strengths. Because of such factors, integration of natural biomolecules on a single hardware is problematic. Since MIP preparation is done in flexible conditions and different monomers can be used, polymers with almost equal operational conditions (pH, solvent, temperature, etc.) can be prepared for a large number of template molecules.

Using molecularly imprinted polymers as conventional photoresist materials also brings additional advantages. Molecularly imprinted polymers can be immobilized to the detector surface as spots using masks and photopolymerization. The compatibility of molecularly imprinted polymers with very small sensors increases the possibility of using these materials in MIP-based multi-sensors.

One of the last but not least important properties of molecularly imprinted polymers is that they can be prepared for practically any compound. Inorganic ions, drugs, nucleic acids, proteins and even cells are template molecules from which MIP preparation is possible. Antibodies can also be produced for a large number of compounds, but these molecules have two main disadvantages when compared to molecularly imprinted polymers.

First, these small molecules must be derivatized in order to produce antibodies for small molecules. This requirement requires the addition of many digits, and these additional operations often significantly change the recognition properties. Second, flexibility in antibody preparation is limited to 20 natural amino acids. However, in the preparation of MIP, there are many monomers that make it possible to design a flexibility and diversity of binding sites that cannot be compared with natural compounds.

MIP-based surface plasmon resonance sensors

Molecularly imprinted polymers can be used as selective recognition elements in the preparation of SPR sensors. For this purpose, the polymer is prepared on the metal film and SPR is used to determine the refractive index

changes that occur on the polymer surface in the presence of the analyte. Although SPR is a highly sensitive system, the applications of molecularly imprinted polymers with SPR are limited. The main reason is that the detection of the refractive index changes caused by the small imprinted molecules is difficult and this reduces the analytical performance. Despite all these limitations, studies have been conducted showing the potential of these materials to be used as recognition elements in SPR systems.

One of the first studies on MIP-based SPR sensors was Lai et al. (1998) . The molecularly imprinted polymers that were shredded and sieved were attached to the silver film and the layers that would perform the recognition after evaporation were immersed in the sample solution for 60 minutes. The films were dried and shifts in the SPR angle correlated with the analyte concentration. The linear dynamic range reaches up to 6 mg/mL, and the detection limit for theophylline is 0.4 mg/mL. It was determined that the precision of the measurements was limited by the reproducibility of the preparation of the recognition layer and the stability was 3 to 5 days depending on the storage conditions. Eight different compounds similar to theophylline, caffeine and xanthine were used in selectivity studies and high selectivity values were obtained.

Recently, Lavine et al. (2007), on the other hand, a theophylline sensor was prepared by taking advantage of the swelling properties of poly N-(N-propyl) acrylamide polymer in aqueous solution depending on the analyte concentration. For this purpose, theophylline-imprinted nanospheres (300 nm in diameter) were prepared by suspension polymerization, coated on gold film and kept on the surface by electrostatic interactions. Even at concentrations as low as 10-6 M, theophylline caused a detectable particle swelling. This behavior is explained by the increase in the transition temperature of the polymer due to the increase in the hydrophilicity of the polymer network, that is, the percentage of water in the polymer. The swelling of the particles was not affected by the ionic strength and the response time was 10 minutes. However, there were problems in the adhesion of the particles on the gold surface. In addition, repeated swelling and shrinkage events reduced the durability and reuse of the sensor.

Kugimiya and Takeuchi (2001) prepared a sialic acid suppressed SPR sensor for the determination of sialic acid containing ganglioside GM1(GM1) in aqueous solution. The resonance angle changed linearly in the concentration range of 0.1-1 mg/mL GM1. No signal could be obtained in the presence of galacturonic acid or sialic acid, which is used as a reference sugar due to its low molecular weight. Sialic acid could only be determined at constant concentration in the presence of GM1 (1 mg/mL) by competitive adsorption. It has been determined that the MIP-based SPR sensor prepared with the study is 200 times cheaper than the SRP biosensors prepared with selective lectins.

Li et al. (2002) prepared a MIP-based SPR sensor for L-phenylalanine methyl ester to monitor binding and elution in situ. However, in this study, very high concentrations of samples (1 g/L) were needed for an observable change in the SPR signal.

Taniwaki et al. (2003) showed how convenient and easy the SPR technique is to study molecular interactions, by using polysulfone together with a glutoamyl residue-derived oligopeptide as imprinting material. By using 9-Ethyladenine as template molecule, the affinity constant depends on the suppression factor.

As a result, they have successfully prepared an SPR sensor ranging from 1.3x104 to 1.6x104 L/mol.

Raitman et al. (2003, 2004) prepared a MIP-based SPR sensor for the analysis of β-nicotinamide adenine dinucleotide (NAD+), β-nicotinamide adenine dinucleotide phosphate (NADP+) and NAD(P)H, the reducing state of these molecules, using polyacrylamide-polyacrylamidophenylboronic acid copolymer. In order to increase the adhesion of the molecularly imprinted polymeric film, a cystamine monolayer was first formed on the gold surface and acrylic acid was covalently bonded. Highly selective binding sites have been obtained by covalent and non-covalent interactions with functional monomers. Interestingly, the resonance angle decreased with increasing analyte concentration. This behavior was thought to be due to the swelling accompanying the binding of the substrate. NAD(P)+ and NAD(P)H cofactors could be determined in the range of 10-6 to 10-3. It has been determined that the prepared sensors are stable for a period ranging

from 2 to 10 days, depending on the daily operation. The selectivity is excellent and the sensor is able to distinguish between NAD(P)+ and NAP+ molecules.

Lotierzo et al. (2004) prepared a MIP-based SPR sensor for the marine toxin domoic acid. Horse radish peroxidase-labeled-domoic acid was used because domoic acid is too small a molecule to cause a detectable refractive index change. The detection limit for domoic acid was determined as 5 μg/L, which is 3 times better than that obtained with the sensor prepared with monoclonal antibodies. In addition, it has been determined that while monoclonal antibodies lose their activity after five reuses, MIP sensors can be regenerated, reused at least 30 times, and are durable for 3 months when stored at 4 0C.

Matsui et al. (2005) prepared a MIP-based SPR sensor embedded in gold nanoparticles. In the study, it was determined that the signal intensity was much higher than the MIP-SPR sensor without gold nanoparticle embedded. During analyte binding, the MIP swells and the distance between the gold nanoparticles and the film on the sensor surface increases. This causes an increase in the severity of the shift in the SPR angle. In this study, a dopamine sensor was prepared and signals could be obtained even at nanomolar analyte concentrations. However, a similar study and comparison has not been made with the unimprinted polymer.

Similarly, Tokavera et al. (2006) prepared an ultra-thin MIP/SPR nanosensor for cholesterol analysis using gold nanoparticles. Charge density vibrations surrounding metal nanoparticles, called localized surface plasmon resonance, are highly sensitive to changes in the environment. The spectroscopic method developed using this principle is transmission surface plasmon resonance spectroscopy (T-SPR). Cholesterol binding to the recognition sites of MIP caused a change in the reflection of all sensor layers on the surface and the T-SPR absorption maximum shifted by 56 nm. Lower values were obtained for similar compounds, stigmasterol (14 nm), digitoxigenin (26 nm), and progesterone (30 nm). However, unfortunately, as in the other study, no study has been done on the non-imprinted polymer.

Devanathan et al. (2005) prepared a sensor capable of detecting subpicomolar levels of δ-opiod G-protein coupled receptor antagonist (DPDPE), a synthetic cyclic enkephalin analogue specific opiate drug. Detection was performed

by plasmon-wave-guided resonance spectroscopy based on the principle of shifting bonding to larger angles, indicating an increase in refractive index and increased film thickness in both s- and p-polarized light. Due to the DPDPE binding, the spectral shifts obtained using the s-polarization are of lower value than the shifts obtained using the p-polarization. This showed that there is a structural anisotropy in the polymer matrix, that is, the binding sites are not randomly oriented. The affinity values obtained are much better than the affinity values of natural receptors, thanks to cooperative multivalent interactions such as hydrogen bonding, electrostatic and hydrophobic interactions between the MIP and the template molecule. No signal was received with the unimprinted polymer. Binding occurred in as little as 3 minutes, but the regeneration time was quite long (several hours). However, the polymeric film can only be used once. The loss of functionality after regeneration was explained by the recognition disruption of molecular gaps in the thin film and the release of specific ligand.

Huang et al. (2006) have prepared an SPR sensor that can perform reliable analysis with very little sample volume. A microfluidic system was used in the study. In the μM concentration range, progesterone, cholesterol and testosterone were analyzed with both the classical SPR and the microfluidic SPR system, and the performance of these two systems was compared. In particular, cholesterol and progesterone were analyzed in real samples and without any concentration. When using the microfluidic SPR/MIP system, higher association rates were achieved due to better interaction of the analytes and the surface containing the recognition sites. In addition, sensitivity comparable to the conventional SPR system was achieved and less sample volumes could be worked with. Unfortunately, it has not been studied with non-imprinted polymers. The same scientists also conducted a study with the multi-channel microfluidic system in which array-MIP film was formed, and showed that many samples could be analyzed simultaneously with a high separation power (Lee et al. 2006).

Banerji et al. (2006) prepared a MIP-based SPR sensor with a polymer prepared by cross-linking polyallylamine in the presence of glucose phosphate for the determination of glucose in urine. The response range of the prepared sensor covers the physiologically important level and thus, there is no need for a preliminary treatment (1-20 mg/mL). However, different analyte binding properties

were observed in different regions of the sensor. In a different approach, gold nanoparticles were embedded in the MIP and a 10-fold increase in the SPR signal was achieved. The presence of nanoparticles provided a more homogeneous surface, but caused the regeneration time to be prolonged from 5 minutes to 50 minutes.

Li and Husson (2006a) prepared a MIP-based SPR sensor using atom transfer radical polymerization and investigated the adsorption kinetics and binding isotherms of amino acids densified with this sensor. The preparation of the polymer by grafting directly on the gold surface eliminated the formation of a very homogeneous film whose thickness can be adjusted, and thus the interlayer diffusional mass limitation that may occur in SPR studies. It has been determined that the binding capacity of MIP surfaces is higher than that of non-imprinted surfaces. When similar template molecules were used, it was observed that the selectivity decreased and was pH dependent. With this study, it was concluded that pH change is effective in adjusting the selectivity in aqueous MIP systems containing ionizable analytes and/or monomers. The same scientists developed two new procedures for the preparation of molecularly imprinted monolayers that allow to control the surface density of the binding sites (Li and Husson 2006 b). With this new approach, template molecules with low binding energy can be suppressed on the gold surface and the response time can be shortened once more. The limitations to be eliminated in these methods are listed as follows: (a) The recognition mechanism is based only on size and shape. There are no mold-specific functional binding sites. Therefore, non-specific binding can be high, (b) Storage time is very short. Within 10 days of surface preparation, 30-40% of the bonding capacity is reduced, most likely due to disruption of the structure of the shape-specific binding sites.

Slinchenko et al. (2004) prepared for the first time a MIP-based SPR sensor that allows to examine the binding kinetics and interaction of ds-DNA without the need to disrupt the natural structure of DNA. In the preparation of the imprinted polymer, 2-vinyl-4,6-diamino-1,3,5-triazine functional monomer, which can hydrogen bond with the A-T base pair of DNA, is used in low concentration so that the template molecule can be easily removed from the structure, and N,N'-methylenebisacrylamide is a crosslinker. was used as Binding of synthetic ds-DNA

identical to the verotoxin gene sequence to the imprinted polymer was analyzed by fluorescence spectroscopy using FTIC-labeled dsDNA, and binding kinetics were analyzed by SPR. The sensor was used for verotoxin ds-DNA analysis in the concentration range of 1-10 nM. Recognition experiments were performed with oligo(dG)-oligo(dC) DNAs and it was determined that the selectivity of the prepared molecularly imprinted polymer was high since a very low shift value was observed in the resonance angle.

The original molecular recognition materials for MIP synthesis, poly[(2-oxo-1,3-dioxolan-4-yl)methylmethacrylate-co-acrylonitrile and a commercial synthetic polyamide-imide polymer (Torlon® 4000T) were used to form films on the SPR sensor surface (Yoshikawa et al. 2005 a,b). The first material, Ac-T-trp and Ac-L-trp, was used as template molecule to prepare MIP capable of chiral separation. Torlon® 4000T was used in a study where 9-ethyladenine was the template molecule. With the obtained high affinity constants, selectivity and low non-specific binding values, these new materials were found to be suitable for MIP preparation. Yu and Lai (2005) prepared a MIP-based SPR sensor for the analysis of mycotoxin ochratoxin A in grain and wine extracts using the electropolymerization method. The prepared sensor showed a linear correlation in the concentration range of 0.05-0.5 mg/L. The detection limit was determined as 0.01 mg/L.

Recently, Matsunaga et al. (2007) prepared an SPR sensor based on lysozyme-selective MIP. The study showed that the presence of NaCl in the prepolymerization mixture (40 mM) and backlink buffer (20 mM) significantly reduced nonspecific binding. In the presence of NaCl, stable conformations of the functional monomer interacting with lysozyme are formed, and thus homogeneous MIPs with only lysozyme-specific binding sites can be prepared. However, increasing the NaCl concentration in the back-binding buffer above 20 mM reduces not only the weak and non-specific binding, but also the affinity of the specific binding sites to lysozyme. Finally, in the study, it was determined that NaCl increased the selectivity in the presence of proteins with similar molecular weights, such as cytochrome c.

Uzun et al. (2009) prepared a hepatitis B surface antibody imprinted poly(hydroxyethyl methacrylate-N-methacryloylchloride-L-tyrosine methylester) (PHEMAT) film on the SPR chip surface to develop a sensor for antibody detection in human blood. The maximum detection limit was 208.2 mIU/mL, and KA and KD values were calculated as 0.015mIU/mL and 66 mL/mIU, respectively. Control experiments of the SPR chip were performed using non-immunized antibody negative serum. Experimental results showed that there was no remarkable sensory response to antibody negative serum.

CHAPTER III

Methodology

Materials

The gold SPR chips were supplied by GWC Tech (Madison, USA) (Product code: SPR-1000-050, Chip gold thickness: 50 nm, Chip dimensions: 1 mm x 18 mm x 18 mm, n_{glass}: 1.72). SIGMA-ALDRICH provided CTX, docetaxel (DTX), methylprednisolone (MP), dexamethasone (DEX), functional monomer methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 2-hydroxyethyl methacrylate (HEMA), azobisisobutyronitrile, sodium chloride, and 2-propene-1-thiol. Merck KGaA, Darmstadt supplied all solvents. Ultrapure water (18.2 MΩ-cm) was used in all solution preparation and dilution processes throughout the experiments.

Design of CTX MIP Sensor

Before the preparation of the CTX imprinted (CTX MIP) and non-imprinted (NIP) sensor, the surface of the chips was cleaned with an acidic piranha solution. They were rinsed with an aqueous ethanol solution and dried at room temperature. After cleaning the gold chip surface, 3 mL of 2.0 mM 2-propene-1-thiol solution was dropped dropwise to form allyl groups. The prepared chip was incubated for 2 h, and unbound 2-propene-1-thiol was removed by washing with ethanol. The chip was then dried in a vacuum oven. CTX MIP sensor was prepared in three stages. The initial step was to modify the sensor surface using allyl. Preparing the CTX-MAA pre-complex was the second stage. Finally, the CTX MIP sensor was created under controlled environments by combining the pre-complex and polymerization mixtures on the modified chip surface (Abu Shama et al.,2022).

The UV spectrophotometer (Thermo Scientific Genesys 10S UV-Vis) was used to analyze the different (1/0.5, 1/1, 1/2, and 1/3) concentration ratios of the CTX-MAA complex to determine the optimal stoichiometric ratio of CTX and MAA pre-complex. The most excellent absorbance value was reported in the CTX-MAA acid combination at a 1/3 ratio. As a result, the CTX: MAA ratio was set at 1/3.

A polymerization solution containing HEMA, EGDMA, CTX-MAA complex in a 1/3 ratio, and azobisisobutyronitrile as initiator was created to prepare CTX MIP nanofilm on the SPR chip surface. The solution was then aliquoted and dropped on the allylated gold surface of the SPR chip. UV light was employed at 25°C (100W, 365 nm) for 60 min (Figure 1B). MAA was mixed into the polymerization system without CTX to produce the NIP sensor rather than the CTX-MAA complex. Finally, the CTX MIP SPR and NIP sensor were washed in an aqueous ethanol solution, dried in a vacuum oven, and stored in a desiccator. Furthermore, CTX was removed from CTX imprinted nanofilm-coated chips using 0.5 M NaOH solution. The NIP sensor was produced using the same procedure without the addition of CTX template analyte molecule (Kurç and Türkmen, 2022).

Characterization Studies

Atomic force microscopy (AFM, Nanomagnetics Instruments, UK), ellipsometer (Nanofilm EP3, Germany), and contact angle (CA, KRUSS DSA100, Hamburg, Germany) measurements were used to characterize the CTX MIP and NIP sensors. To investigate the depth of the surface, an ambient AFM was used in tapping mode. Three-dimensional images were obtained by scanning the surface of the plasmonic sensors with high resolution. While the scanning speed of the images was 1 μm/s, an image was obtained from an area of 1 × 1 μm^2. In addition, the thickness of the polymeric layer on the gold surface of the SPR chip was measured using an auto-nulling imaging ellipsometer. Finally, the contact angles of CTX MIP and NIP sensors were measured. During the process, the sessile drop method obtained the contact angle values, and the wettability was measured by dropping water on the surfaces. After dropping water into three different regions, images were taken in each region, and contact angles were determined (Yılmaz et al., 2022).

Kinetic Studies of CTX MIP Sensor

Kinetic analyses of CTX MIP and NIP sensors were performed using an SPR imager II (GWC, Madison, USA) with a flow rate of 150 μL/min and an operating wavelength of 800 nm. CTX was detected from the aqueous solution and artificial plasma samples by the CTX MIP sensor. Firstly, CTX detection studies in varied aqueous mediums at different pH (5, 6, 7.4, 9) were evaluated to establish

the effective pH for the detection of CTX in the 0.05-150 µg/mL range. The detection time was nearly 10 minutes. The CTX MIP sensor was equilibrated with 0.5 M phosphate buffer at pH 6.0. After CTX adsorption on the sensor surface, 0.5 M of NaOH solution achieved desorption. The fluctuations in resonance frequency were tracked in real-time and reached a steady-state equilibrium in 15 min. The aqueous solutions of DTX, MP, and DEX were used to test the selectivity of the CTX MIP sensor. The reusability of the CTX MIP sensor was evaluated by cycling equilibration-adsorption-desorption four times with CTX solutions containing 20.0 µg/mL in water.

Confirmation Analysis of CTX MIP Sensor

To test the reliability and validity of the constructed SPR sensor, the experiments were carried out using artificial plasma. A Dionex Ultimate HPLC system with a photodiode array detector and a C18 (100 mm 4.6 mm i.d., 5 µm particle size) column was kept at 25 °C to achieve chromatographic separation. By carefully weighing 25 mg of CTX into a 25 mL volumetric flask containing a mobile phase, a stock solution of CTX (1000 µg/mL) was produced. Working standard solutions were made daily from the mobile phase-infused stock solution by filtering them through a 0.45 µm membrane filter before injection. 1000 mL of water was used to dissolve 6.8 g of potassium dihydrogen phosphate, and 10 M potassium hydroxide was used to bring the pH level down to 5.0. Isocratic elution was carried out using phosphate buffer and acetonitrile (50/50, v/v). The flowing rate was 1 mL/min, and the length of operation was 10 min. The sample was injected into the HPLC system at a volume of 20 µL and 230 nm wavelength (Thomas et al., 2012).

CHAPTER IV

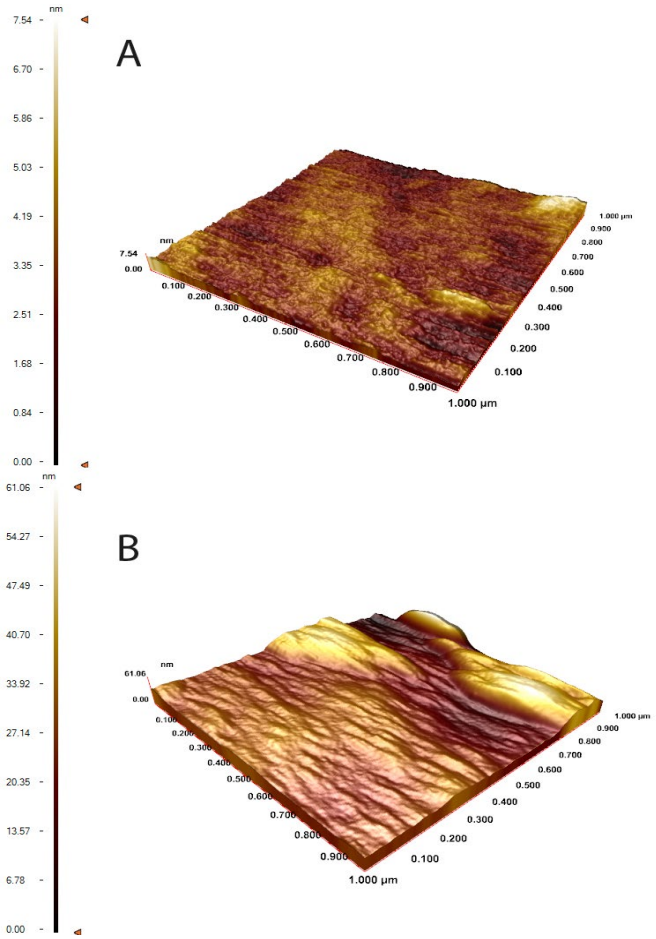
Result and Discussion

Characterization of CTX MIP Sensor

AFM in a half-contact mode characterized the bare chip and CTX MIP sensor surface morphology. Figure 14 shows AFM images of the bare surface and CTX MIP sensor. AFM images determined the surface depth of the bare chip to be 7.54±2.17 nm, while the surface depth of the CTX MIP sensor was 61.06±1.0 nm. The difference in surface depth values between the bare chip and the CTX MIP sensor indicates that the polymeric structure was successfully fabricated on the chip surface.

Figure 14:

AFM images, A) Bare Chip, B) CTX MIP sensor surface.

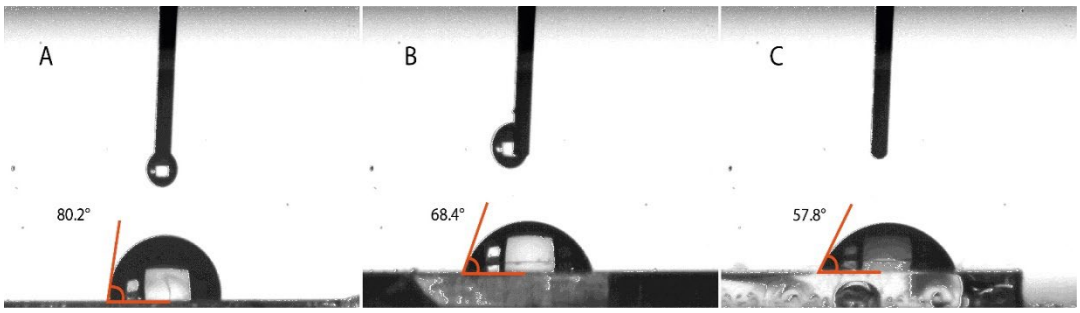


The thickness of the SPR sensor surfaces following each modification step was measured using ellipsometry. According to the results, the thickness of the unmodified and CTX MIP sensor surfaces were 8.9±0.6 nm and 65.5±0.9 nm, respectively. These outcomes matched those of the AFM. The findings of the AFM and ellipsometry measurements demonstrated the rough surfaces of the CTX MIP sensor surfaces, and the SPR sensor surfaces' thickness differences demonstrated the imprinting process's efficacy.

The CA images of the bare chip surface, the ally-modified chip surface, and CTX MIP sensor surface are given in Figure 15. According to the results of the CA measurements, the CA value of the surface of the bare chip was recorded as 80.2°±1.5, while the contact angle of the ally-modified chip was measured as 68.4°±2.3. After CTX imprinting, the wettability of the CTX MIP sensor surface increases according to the contact angle value measured as 57.8°±1.2.

Figure 15:

CA measurement, A) Bare chip, B) The ally-modified chip, C) CTX MIP sensor.



Aqueous Solution Study of CTX MIP Sensor

Effect of pH and Imprinting Factor

The molecular imprinting method relies on the interaction of a functional monomer and a template to produce a complex, where a three-dimensional polymer network is created following the construction of this complex and the application of a cross-linking agent. When the template is removed from a polymer, it leaves behind specific recognition sites structurally, dimensionally, and functionally identical to the template molecule. Intermolecular interactions such as dipole-dipole interactions, hydrogen bonds, and ionic interactions between the template molecule and functional groups in the polymer matrix frequently drive the molecular

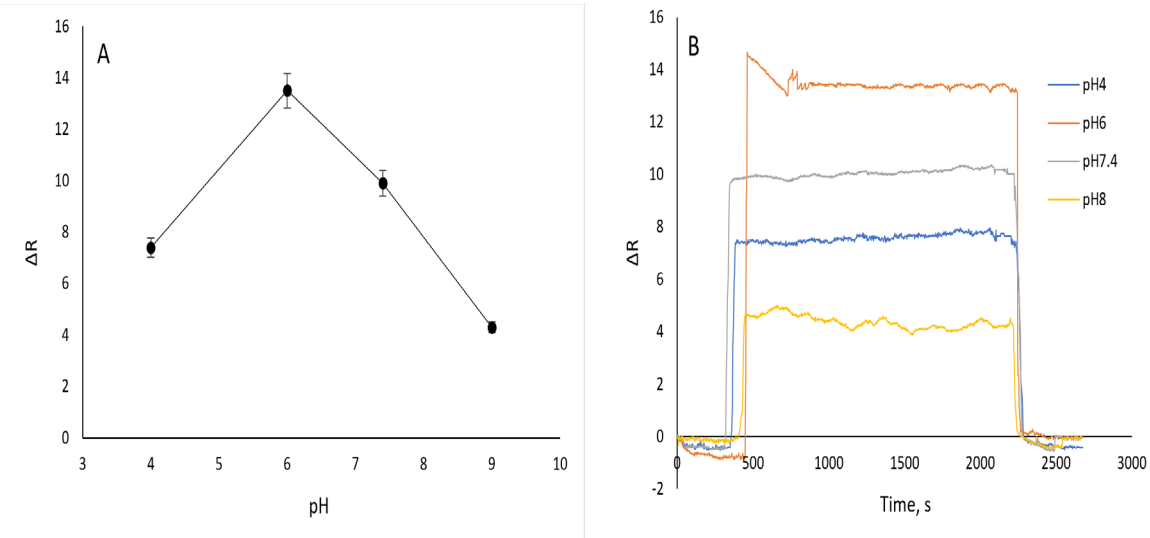
recognition phenomenon. As a result, only the molecules from the template are recognized and bound by the resulting polymer. This recognition and binding strictly depend on the experimental conditions; one of the crucial ones is pH, which affects the structural properties of both the cavities and the template molecule.

Figure 16 shows the impact of pH (4.0, 6.0, 7.4, and 9.0) on the adsorption of CTX to the polymer formed on the surface of the CTX MIP sensor. All tests and measurements were conducted three times, with the average data (Figure 15A and 15B). The highest CTX adsorption took place at pH: 6.0, as shown by the graph we obtained from the sensorgram. The interaction between CTX-imprinted polymers relied on hydrogen bonds. The MAA hydroxyl group (O-H) and CTX would not form hydrogen bonds because of the deprotonation impact of high pH, and the hydrogen bonding increases in low pH because of protonation, afterward at lower pH because of the saturation of binding sites also hydrogen binding decreases.

As a result, the sensor's selectivity was decreased, and ligand binding affinity to the template molecule varied with pH levels. The pH effects results' relative standard deviation (RSD) was less than 1.29, showing repeatability.

Figure 16:

A) Sensorgram responses with error bars, B) Sensorgrams of CTX by designed CTX MIP sensor in different pH (C: 20.0 µg/mL, T: 25.0°C, repeated three times (n = 3)).



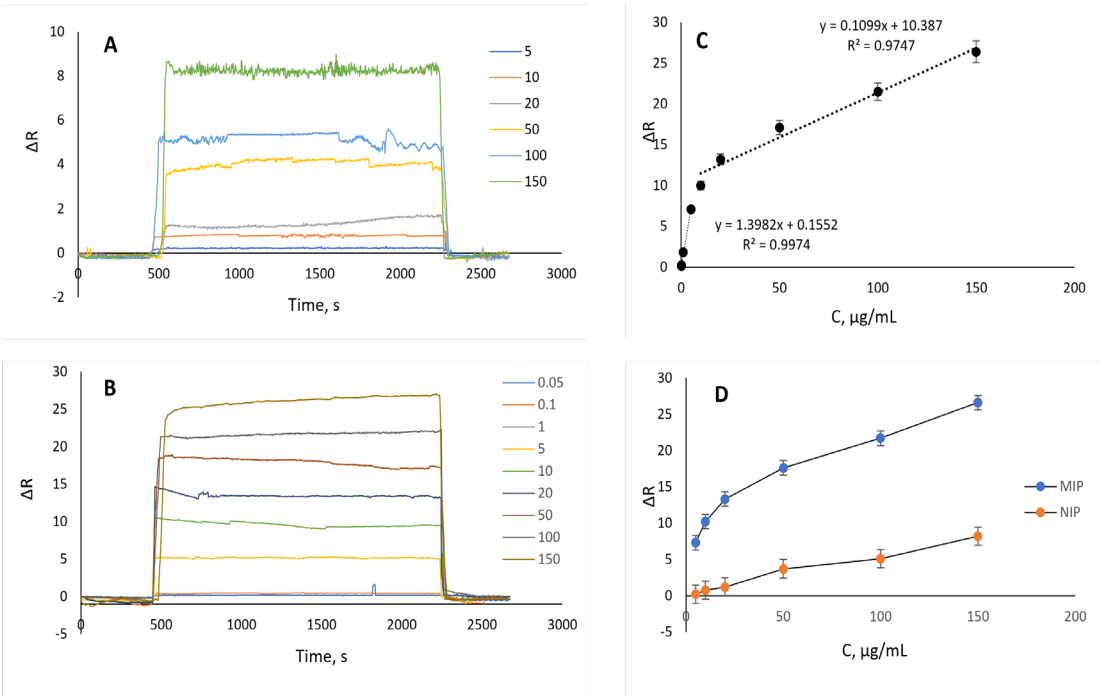
Effect of CTX Concentration

In Figure 17, we compared the NIP sensor and CTX MIP sensor with a range of CTX concentrations (5 µg/mL-150 µg/mL for the non-imprinted sensor and 0.05–150 µg/mL for CTX imprinted sensor) to assess the impact of imprinting on CTX adsorption and to estimate kinetic parameters. CTX imprinted polymer-coated SPR nanosensor chips were used to conduct real-time CTX adsorption tests. The investigation was conducted with a CTX concentration range of 0.05 µg/mL to 150.0 µg/mL. To equilibrate the sensors, we employed a phosphate buffer with a pH of 6.0. The SPR view program then computed the outcomes after delivering the solutions to the SPR sensor. It took 40 min to complete the adsorption, desorption, and regeneration procedures. Figures 21 (B) and 21 (C) show the sensorgrams we obtained and the calibration curves we drew. The measurements for the limit of detection (LOD) and limit of quantification (LOQ) were calculated using the 3 s/m and 10 s/m approaches. The results showed that the LOD and LOQ values were 0.012 and 0.036 µg/mL, respectively.

In the range of concentrations of 0.05-150 µg/mL, the correlation coefficients for the CTX MIP sensor are y = 1.3982x+0.1552 with 99.7% accuracy in the concentration range of 0.05-5 g/mL and y = 0.1099x+10.387 with 97.5% accuracy in the concentration range of 10-150 µg/mL. In the range of concentrations of 0.05-150 µg/mL, the correlation coefficients for the CTX MIP sensor are y = 1.3982x+0.1552 with 99.7% accuracy in the concentration range of 0.05-5 µg/mL and y = 0.1099x+10.387 with 97.5% accuracy in the concentration range of 10-150 µg/mL. The analysis of these compounds at low concentrations was particularly crucial since CTX, and other anticancer medications had insufficient quantities in human fluids and ambient waters. As seen in Figure 21 (D), the non-imprinted polymer-coated chips' surface adsorption was determined to be 8, with the functional groups and CTX forming most hydrogen bonds. On the other hand, molecular imprinting improved the adsorption of CTX on the sensor surfaces, as indicated by the surface adsorption of 27 for the CTX MIP sensor. These analyses determined the imprinting factor (IF) to be: $\Delta R(MIP)/ \Delta R(NIP) = 27/8 = 3.4$. As a result, the affinity to the CTX molecule was raised by 3.4 folds, indicating that imprinting was effective.

Figure 17:

(A) NIP sensor response for CTX with a range of 5–150 µg/mL concentrations. (B) CTX MIP sensor response for CTX with a range of concentrations of 0.05–150 µg/mL. (C) CTX MIP sensor linear response for CTX with a range of concentrations of 0.05–5 µg/mL and 10–150 µg/mL. (D) Comparison of CTX MIP and NIP sensor response for CTX with a range of 5–150 µg/mL concentrations.



Selectivity

Despite their exceptional sensitivity to the target molecule, one of the essential characteristics of the sensors was their extremely low selectivity for other compounds in the environment. Selectivity tests involving the addition of other chemotherapy drugs that are expected to be present in the medium were conducted to assess the sensor's selectivity.

We employed DEX, MP, and DTX, three different drugs, for our selectivity investigations. The target molecule's close structural resemblance determined the selected molecules and their likelihood of existing in the same surroundings. DTX is also a taxoid, antineoplastic agent, and its structural analog to CTX is used to treat prostate cancer similarly. MP and DTX are administered to reduce the adverse effects of treatment during the treatment period. They reduce allergic reactions and

other auto-immune responses (Zhi et al.,2018). Because of their usage during the treatment period, they may be in the biological fluid of the patient and analytical matrix, which may obstruct the analysis of CTX, so we choose them to investigate. Each drug was administered to the sensor at 20.0 µg/mL. Figure 22A and 22B present the obtained sensorgram, while Table 1 contains the calculated values.

As shown in the table below, CTX's calculated selectivity (Equation 1) constants for DTX, MP, and DTX were 7.8, 5.4, and 3.1 (Figure 22A and Table 3), respectively. For non-imprinting sensor, these values were 0.26, 0.38, and 0.43 (Figure 22B and Table 1). The imprinting efficiency of CTX for DTX, MP, and DTX was demonstrated by the computed relative selectivity (Equation 2) constants of CTX (30.4, 14.2, and 10.5). As a result, CTX had a better response signal from the imprinting sensor than other drugs.

$$k = \Delta R_{template} / \Delta R_{competitor} \qquad 1$$

$$k' = k_{MIP} / k_{NIP} \qquad 2$$

Figure 18:

Selectivity Study, **A)** CTX MIP sensor selectivity (C_{CTX}, C_{DEX}, C_{MP} and C_{DTX} eaqual to 20.0 µg/mL, T: 25°C, pH: 6), **B)** NIP sensor (C_{CTX}, C_{DEX}, C_{MP} and C_{DTX} eaqual to 20.0 µg/mL, T: 25°C, pH: 6).

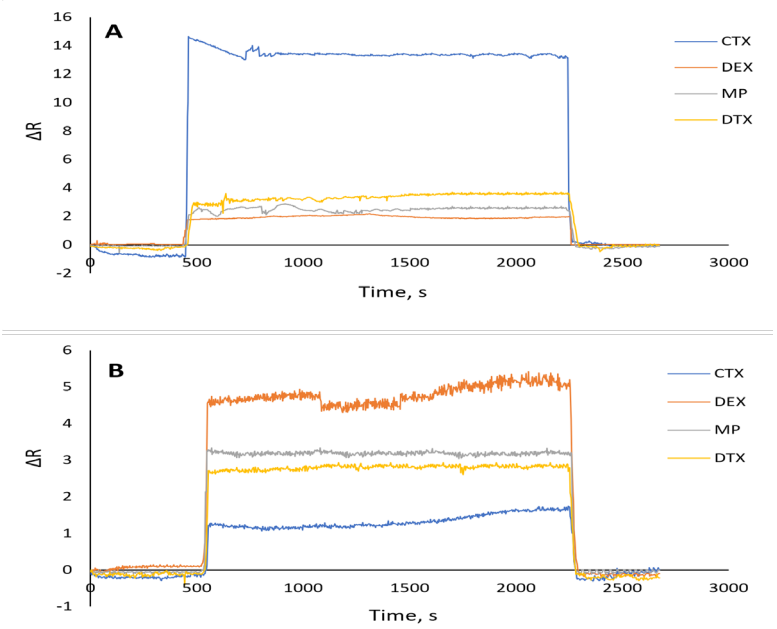


Table 1: Selectivity coefficient of CTX MIP sensor.

	CTX MIP sensor		**NIP sensor**		k′
	ΔR	K	ΔR	K	
CTX	14		1.2		
DEX	1.8	7.8	4.7	0.26	30
MP	2.6	5.4	3.2	0.38	14.2
DTX	3.1	4.5	2.8	0.43	10.5

Adsorption characteristics and isotherms

We determined the kinetic parameter using the equation given in references (Zhi et al.,2018). Table 2 below contains the calculated parameters. The target molecule's interaction with the developed sensor and the magnitude of the connection was revealed by the rate constants and equilibrium constants of the adsorption of CTX by the sensor. Table 2 shows that both the association rate constant (0.85 mL/µgs) and the association equilibrium constant (2.02 mL/µg) were more significant than the dissociation rate constant (0.42 1/s) and dissociation equilibrium constant (0.5 µg/mL), respectively. Results demonstrate the high affinity of CTX for the sensor of interest.

Table 2: Kinetics constants for CTX MIP sensor.

Association Kinetics Analysis		**Equilibrium Analysis (Scatchard)**	
k_a (mL/µgs)	0.85	ΔR_{max} (µg/cm^2)	23
k_d (1/s)	0.42	K_A (mL/µg)	0.084
K_A (mL/µg)	2,02	K_D (µg/mL)	11.9
K_D (µg/mL)	0.5	R^2	0,91
R^2	0.99		

Table 3: CTX MIP sensor adsorption models.

Langmuir		**Freundlich**		**Langmuir- Freundlich**	
ΔR_{max}:	6.7	ΔR_{max}	14	ΔR_{max}:	19
K_D	0.06	1/n	0.76	1/n	0.76
K_A	16.7	R^2	0.978	K_D	0.045
R^2	0.992			K_A	22.2
				R^2	0.998

Langmuir, Freundlich, and Langmuir-Freundlich isotherm models were employed to ascertain the CTX's adsorption characteristics on the CTX MIP sensor. A detailed explanation of adsorption isotherms is given in our previous article (Türkmen et al., 2021).

Table 3 contains the calculated findings of the adsorption isotherm data. Our data analysis (correlation coefficient and maximum response signal values) revealed that the CTX adsorption feature resembled the Langmuir-Freundlich model. The Langmuir-Freundlich model depicts the behavior of the heterogeneous surface across an extensive concentration range and is appropriate for a system that does not precisely match either system alone.

Repeatability and storability

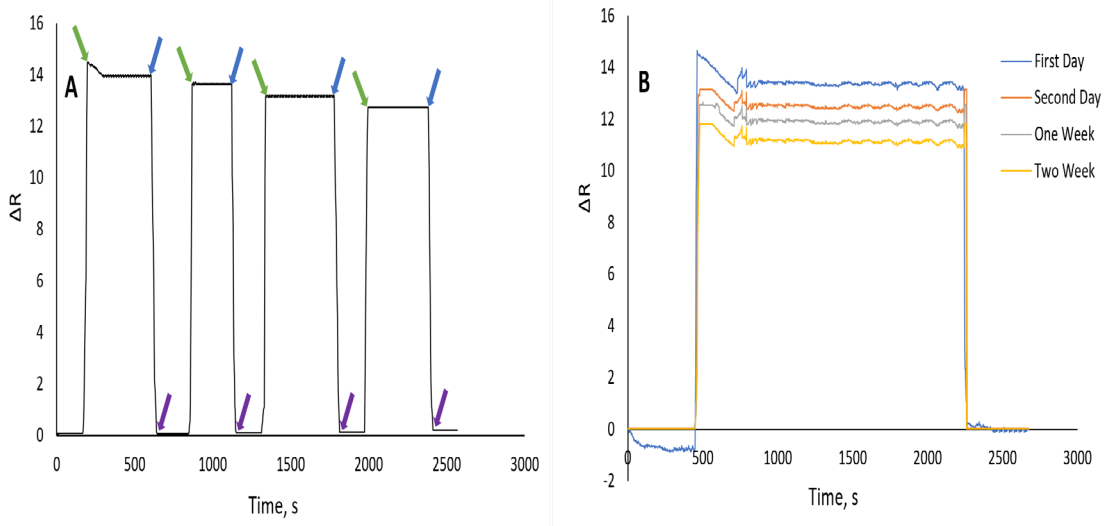
In Figure 19, the repeatability and reusability of the imprinted sensors were examined. For the repeatability experiment, we performed four continuous analytical cycles (repeated three times (n=3)). The CTX concentration was 20.0 µg/mL. The repeatability experiment demonstrated that the CTX MIP sensor retains analysis capacity even after four continuous analysis cycles, performed in Figure 23A.

To test the sensor's storability, we experimented with Figure 23B on several dates spanning from one day to two weeks. After its manufacture, we chose the 1st day, 2nd day, 1st week, and 2nd week (storage condition: pH 6.0 buffer solution, in the refrigerator). The system's reproducibility studies were determined using precision studies.

For intraday testing (five replicates with three groups), studies on the CTX MIPsensor's repeatability of the signal response were statistically analyzed, and reproducibility accuracy was confirmed by computing the percent relative standard deviation (% RSD). Expressed as a percent RSD, the results of intraday trials were reported as being less than 1.3, showing strong repeatability. After two weeks, the data indicated that the sensor maintained a required affinity to CTX. Repeatability and storability were examined, and our sensor had excellent repeatability and storability, which was ideal for a sensor because using the same sensor saved money, time, and human labor.

Figure 19:

Repeatability and storability study. **A:** Repeatability, to ensure consistency, the experiment was carried out three times (n = 3) with five different duplicates (green arrow: equilibration, blue arrow: adsorption, purple arrow: regeneration). **B:** Storability, CTX MIP sensor sensorgrams obtained different dates. *(All repeatability and storability studies were repeated three times (n = 3), C_{CTX}: 20.0 µg/L, t: 25°C)



Confirmation Analysis in Artificial Plasma

The HPLC system confirmed the selective determination of CTX in artificial plasma samples using the CTX MIP sensor. The proposed HPLC method was validated per The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. Different concentrations of CTX solutions (0.1-150 µg/mL) were prepared and given to the HPLC system to create a calibration curve. It was decided to compare the results from the SPR method with those from the HPLC method at this point. It was decided to compare the results from the SPR method with those from the HPLC method at this point. The recovery values obtained from the SPR study show parallelism with the data obtained from HPLC. CTX demonstrates linearity over the concentration range from 0.1-150 µg/mL (Table 4). $y = 28550x + 4278.7$ ($R^2 = 0.9999$) was found to be the linear regression equation. The LOD was determined to be 0.0305 µg/mL, whereas the LOQ was determined to be 0.0840 µg/mL. Compared to the HPLC method, the SPR method does not require preliminary preparation, does not require column conditioning, and is easier to implement. An excellent correlation was found between the two analytical techniques ($R^2 = 0.9999$ for HPLC and $R^2 = 0.9974$ (0.05-5 µg/mL) and $R^2 = 0.9747$ (10-150 µg/mL) for SPR). In light of the data obtained, it can be said that the SPR method is reliable.

Table 4: Accuracy of CTX MIP sensor method compared to HPLC method.

Prepared concentration (µg/mL)	Found (µg/mL)		Recovery (%)	
	SPR	HPLC	SPR	HPLC
0.05	0.049	Na	97	Na
0.1	0.099	0.097	99	97
1	0.098	0.098	98	98
5	4.96	4.97	99	99
10	9.97	9.96	99	99
20	19.96	19.94	99	99
50	49.97	49.95	99	99
100	99.92	99.90	99	99

150	148.6	148.9	99	99

CHAPTER V

Conclusion

For the detection of chemotherapeutic drug, CTX, a molecularly imprinted polymer-based plasmonic SPR sensor, has been effectively synthesized. According to our knowledge, this is the first molecularly imprinted SPR sensor designed for CTX detection. The MAA-EGDMA-HEMA polymer was UV photopolymerized to create the CTX MIP sensor, which AFM, ellipsometry then characterized, and CA measurements. This method allowed for the rapid and simultaneous analysis of CTX with LOD and LOQ values of 0.012 µg/mL and 0.036 µg/mL, respectively, at low detection limits. Additionally, the CTX MIP sensor directly measured CTX with excellent accuracy and selectivity. The Langmuir-Freundlich model, which depicts the behavior of the heterogeneous surface across a wide concentration range, was shown to be the best suitable model for the CTX MIP sensor. It was discovered that our sensor had high repeatability and storability—an important feature for any sensor because reusing the same sensor meant less money and time spent developing the sensor. The HPLC system validated the SPR nanofilm sensor's specific determination of CTX in the artificial plasma sample. The SPR method is simpler to use and requires less previous preparation than the HPLC method, as well as no column conditioning. The two analytical methods showed excellent agreement (R^2 = 0.9999 for HPLC and R^2= 0.9974 (0.05-5 µg/mL) and 0.9747 (10-150 µg/mL) for SPR). The results allow it to be concluded that the SPR approach is reliable.

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Appendices

Appendix A

Ethical Approval Document

There is no ethical approval document that can be presented.

Assoc. Prof Dr. Süleyman Aşır
Supervisor

Appendix B

Curriculum Vitae

NAME SURNAME	BUSE UĞUR
DATE OF BIRTH	10/04/1991
NATIONALITY	Turkish Cypriot , Turkish
LANGUAGES	Turkish , English , French

Objectives:

My objective is to join an organization where I can apply my credentials as an engineer and to contribute positively to the success and growth of the company through hard work and dedication.

Education:

2023 Near East University (Nicosia- Cyprus)

Biomedical Engineering (PhD)

2017 Near East University (Nicosia- Cyprus)

Biomedical Engineering (Msc)

2014 Near East University (Nicosia- Cyprus)

Biomedical Engineering (Bsc)

2009 Turk Maarif College (Nicosia- Cyprus)

Science

Experience:

TRNC Ministry of Health

-Work as a Biomedical Engineer

Dr. Burhan Nalbantoğlu Government Hospital, North Cyprus,Turkey. (since November 2017- 2023)

-Work as a Biomedical Engineer

Near East University, Faculty of Engineering, North Cyprus, Turkey (since November 2017-)

-Work as a part-time Lecturer

Near East University,Faculty of Engineering, North Cyprus, Turkey (2016-2017)

-Worked as full-time Lecturer

Near East University , North Cyprus, Turkey (2014-2016)

-Worked as Research Assistant

Skills:

-Device troubleshooting and creative problem solving backround.

-Technically proficient in MsWord, Excell,Power Point ,C programming, SPSS

-Able to work as a part of team or individually.

- Can withstand a pressured team.

- Good communication skills and easy to adapt.

-Competent with dealing with letters, emails and telephone queries.

- Good listener and attention to details

Field of Interest:

-Clinical Engineering

-Service Engineering

-Biomedical Design

-Application Engineering

-Image Processing

-Project Management

-Marketing & Sales

Awards/Honours/Activities:

-Second Ranking Graduate of the Faculty of Engineering February 2014.

-Second Ranking Graduate of the Department of Biomedical Engineering February 2014

-Certificate of High Honours Near East University (7/7 Semesters).

-Terin Adali, Buse Ugur, Nail Ulakbasi, Synthesis of Silk Fibroin-Folic Acid-Fe^{+3}
Nanoparticles For Molecular Imaging, (Poster Presentation, Abstracts Book P70), International Biomedical Engineering Congress 2015, 12-14 March 2015,Near East University, Grand Library, Nicosia, North Cyprus

- Kamil Dimililer, Buse Ugur and Yoney Kirsal Ever Tumor detection on CT Lung Images Using Image Enhancement, ISTEC 2016 (International Science and Technology Conferences), 13-15 July, Vienna, Austria

- Kamil Dimililer, Yoney Kirsal Ever and Buse Ugur, ILTDS: Intelligent Lung Tumor Detection System on CT Images Second International Symposium on Intelligent Systems Technologies and Applications (ISTA'16), September 21-24, 2016, Jaipur, India.

- Selin Uzelaltinbulat and Buse Ugur, Lung Tumor Segmentation Algorithm, 9th International Conference on Theory and Application of Soft Computing, Computing with Words and Perception, ICSCCW 2017, 24-25 August 2017, Budapest, Hungary

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Appendix C

Turnitin Similarity Report