BIOTECHNOLOGY AND MATERIALS SCIENCE

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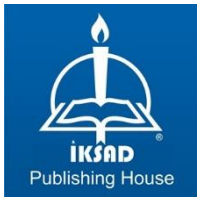

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CONTENTS

PREFACE

Biotechnology is, in essence, the deciphering and use of biological knowledge. It is highly multidisciplinary since it has its foundations in many disciplines, including biology, microbiology, biochemistry, chemistry, and chemical and materials engineering. Similarly, materials science is a broad field and can be considered to be an interdisciplinary area. Included within it are the studies of the structure and properties of any material, the creation of new types of materials, and the manipulation of a material's properties to suit the specific application's needs.

The chapters in this book have various areas of expertise in biotechnology and materials science. Therefore, this book is interdisciplinary and is written for readers with a background in biotechnology, chemistry, nanotechnology, and materials science. I believe that this book will be of interest to university students, lecturers, and researchers interested in these areas.

The book consists of six chapters with independent contents and includes these specific topics; environmental and health effects of textile dyes, molecularly imprinted polymers, biotechnological recovery of rare earth elements, nanomaterials' biomedical applications, single nucleotide polymorphism detection methods in cotton and boron derivatives' biotechnological applications.

Dr. Nimet YILDIRIM TİRGİL

Ankara/2021

BÖLÜM 1

ENVIRONMENTAL AND HEALTH EFFECTS OF TEXTILE DYES CHARGED FROM INDUSTRIAL WASTEWATER

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INTRODUCTION

Wastewater of the textile industry containing a large number of toxic pollutants such as synthetic dyes is ongoing concerning the pollution issue. Textile dyes are extensively utilized in a variety of industrial applications due to their easy usage, low cost of the synthesis process, and be stable (Chang et al. 2001). It is reported that a high amount of approximately 50.000 tons of wastewater containing textile dyes have been released into the aquatic environment (Lewis 1999). Treatment of wastewaters containing dyes is one of the most challenging issues because of requiring a high amount of chemical and biological oxygen (Aksu 2005). The presence of dyes in the aquatic environments is harmful to livings because of having aromatics and chlorides in their structure (Banat et al. 1996; Clarke and Anliker 1980; Fu and Viraraghavan 2001; Mishra and Tripathy 1993; Robinson et al. 2001; Zollinger 1987). Unfortunately, biodegradation of textile dyes is very difficult because of having aromatic molecular structures. Additionally, most of the dyes are known as carcinogens owing to have benzidine and other aromatic compounds.

The classification of dyes is done in different ways such as chromophore groups (Banat et al. 1996; Fu and Viraraghavan 2001; Mishra and Tripathy 1993). For instance, the chromophores are azo or anthraquinone groups in anionic and/or non-ionic dyes. The disruption of azo linkages forms the presence of toxic amines in the textile effluents. The dyes containing anthraquinone groups have more

resistance to degradation because of their fused aromatic structure, therefore they remain for a long time in wastewaters.

Reactive dyes having azo-based chromophores are used in textile industries because of having bright colors, applying with simple techniques, and requiring low energy consumption. On the other hand, the treatment of wastewater containing water-soluble dyes is the most problematic ones due to pass the conventional treatment systems thoroughly (Aksu 2005; Hu 1992; Juang et al. 1997; Karcher et al. 2001; O'Mahony et al. 2002; Robinson et al. 2001; Sumathi and Manju 2000). Also, the basic dyes, are easily visible although they are very low concentrations due to their high brilliance. (Chu and Chen 2002; Fu and Viraraghavan 2001, 2002; Mittal and Gupta 1996). Metal complex dyes are carcinogenic because they are based on chromium (Gupta et al. 1990). Disperse dyes do not show ionization in an aqueous solution (Aksu 2005).

Today, the control of the increasing amounts of dyes in wastewaters is an ongoing problem because of their toxic and carcinogenic potential. Different kinds of wastewater treatment technologies are improved to remove dyes from the textile industry effluents. Among them, biological methods are more advantageous owing to their inexpensive and eco-friendly nature (Davies et al. 2005). For example, fungi are suggested as low-cost biological materials to remove pollutants due to their high growth rate and ability to resist unfavorable environments like low pH, etc. (Aksu et al. 2010). This study is aimed to explain the classification of dyes and review the environmental and health effects

of dyes. And also, it is intended to focus on briefly exploring the potential usage of biological materials towards the decolorization of dyes.

1. TEXTILE DYES

1.1. A brief history of Dyestuffs and Dyeing

In the most ancient times mankind has been trying to add color to life, and primitive people used natural matter to paint their pictures on the ancient cave walls. Primitive man used different pigments such as black, white, reddish, etc. obtained from ochre in cave paintings according to scientists (Orna 2015). Approximately 7,000-2,000 BCE, the human started to use textile products, and also colored these products (Grierson 1989). The utilization of natural colorants like organic textile dyes has a timeless history.

A chemist called William Henry Perkin discovered the first synthetic dye accidentally in 1856. Perkin (1856) studied to produce the drug quinine from aniline finding in the coal chemically. During the experimental period, he obtained a thick dark sludge. Perkin tried to dilute this sludge with alcohol, then established that the color of the solution would turn silk purple (Fox 1987; Garfield 2000). Researchers followed this concept and developed new dyestuff that took part in the markets. In 1865 Kekule discovered the molecular structure of benzene and provided the development of new dyes. At the beginning of the 20th century, synthetic dyes had almost completely replaced natural ones (Welham 2000).

1.2. The Structure of Dyes

Dyes are defined as coloring agents used to color substances by spontaneously or suitably reacting with the material. The appearance of color is the reflection of rays falling on a pigment (Aksu 2005). All aromatic compounds absorb electromagnetic energy but colorful aromatic structures like dye molecules adsorb visible light (350- 800 nm). Dyes contain chromogen groups that provide adhesion of dyes onto and into the fibers. Chromogen groups are aromatic structures with the inclusion of chromophores providing a colorful appearance of molecules. Chromophores are electron systems with conjugated double bonds. Dyes also include oxochromes, which are substituents that intensify their color by changing the energy of the electron system. Usual chromophores and auxochromes are -C=C-, -C=N-, -C=O, -N=N-, -NO2 and NH3, -COOH, -SO3H and –OH, respectively (Zee 2002).

The chromophore, which generates color in an organic compound, must be a part of a conjugated system. For example, as shown in Figure 1, an azo group places between methyl groups produces a colorless compound, but if it is placed between aromatic rings, the orange color is obtained.

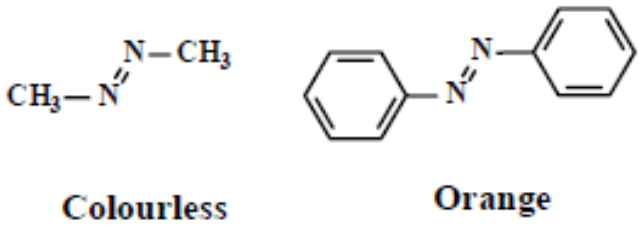

Figure 1: The importance of the placement of chromophore in an organic compound (IARC, 2010)

Auxochromes, which influence the solubility of dyes, are essential ring substituents to provide target colors. Figure 2 shows the effect of auxochromes on the dye molecule's light absorbance property.

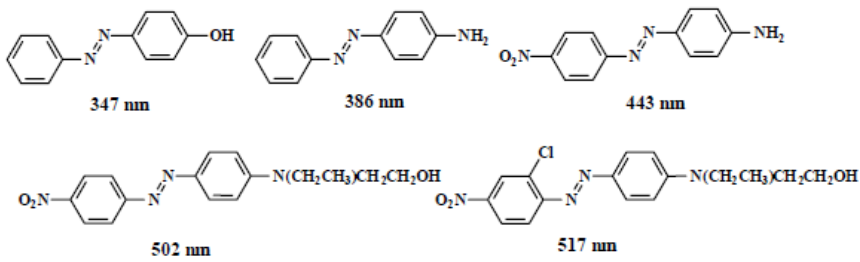

Figure 2: Effects of substituents groups within an azo dye system (Hou et al., 2007)

Approximately about 20-30 different types of dyes can be distinguished depending on the chemical structure of the chromophores. Chromophore groups are divided into 7 groups including Nitroso, nitro, azo, ethylene, carbonyl, Carbon-Nitrogen, Sulfur groups according to their chemical structure (Vandevivere et al. 1998). They contain one or more variable bonds which adsorb light to

ensure the brightness of dye. The most important groups are listed as azo, anthraquinone, phthalocyanine, and triarylmethane dyes. The dyeing process of commercially available azo dyes consists of several components to improve the technique. Acid, Mordant, Reactive, and Solvent dyes can be listed as extensively used azo dyes in industrial applications.

1.3. Classification of dyes

Kirk–Othmer Encyclopedia (Suslick 1998) and *Colour for Textiles* (Ingamells 1993) described the complex structure of dyes. According to the reports of The Society of Dyers and Colourists (1976), more than 8000 dyeing process-related chemical products are found in The Color Index (CI). Dyes are classified with the numbers in Color Index (CI). A five-digit number is assigned for a dye according to the chemical structure. In this classification system, the first and second words are related to the class and the shade of the dye, respectively. For example, the dye Acid Violet 34 is classified as CI 61 710. Two groups of dyes are present as natural and synthetic but in the textile industry utilization of synthetic dyes is more than others due to cost-effectiveness. In this paper, synthetic dyes are discussed in detail because of their negative environmental impacts. Dyes are classified according to their various characteristics such as resolution, chemical structure, and dyeing properties (Kurbanova et al. 1998). Figure 3 shows the classification of dyes.

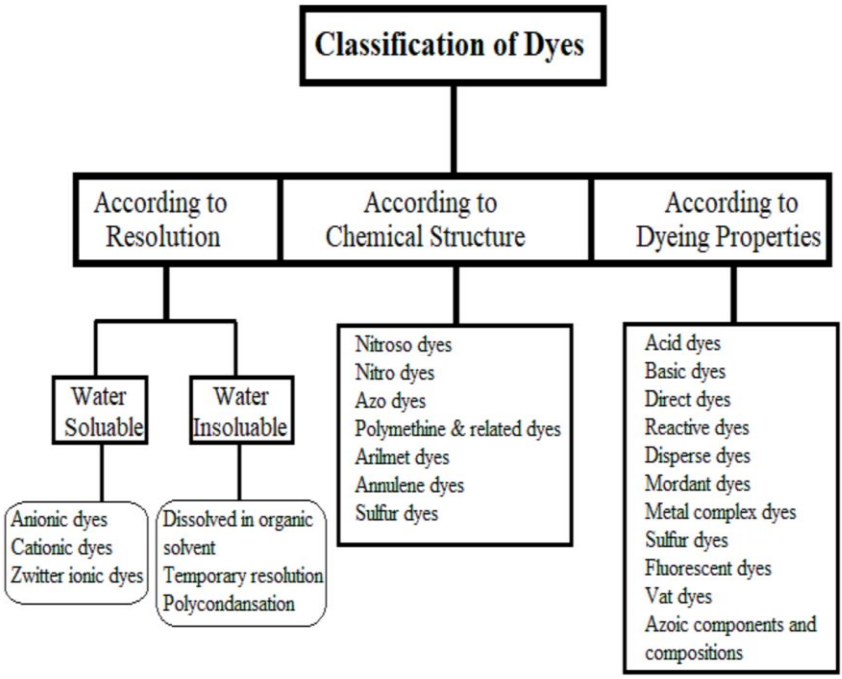

Figure 3: Classification of dyes (Mishra and Tripathy 1993; Kurbanova et al. 1998; Hao et al. 2000; Al Prol 2019)

1.4. Classification of According to Dyes Resolution

According to resolution dyes are classified as water-soluble and insoluble. Water-soluble dyes are divided into anionic, cationic, and zwitterionic. The water-insoluble dyes are grouped as dissolved in the organic solvent and substrate, temporary resolution, polycondensation. Water-soluble dyes contain groups that are capable of salt formation. Cationic dyes have more toxic effects, than toxicity is followed by anionic acid and direct dyes. Basic dyes are also cationic dyes because of their cationic functional groups. Anionic water-soluble dyes include

water-soluble groups as sodium salts (-SO_3Na and -COONa) of mostly sulfonic (-SO_3) and little the carboxylic acid (-COO -) which work as anionic functional groups. The examples of ionic dyes can be classified as Reactive azo, Basic, Direct, and Acid dyes (Anliker et al. 1981). Disperse, pigment and solvent dyes are classified as non-ionic dyes (Hao et al. 2000).

1.5. Classified Dyes According to Chemical Structure

Dyes are divided into 7 groups including Nitroso, nitro, azo, polymethine, arilmet, annulene, and sulfur groups according to their chemical structure. The most important group is the azo dye group which is characterized by having an azo chromophore group (-N=N-). The azo group contained dyes that are synthesized chemically. Natural dyes don't contain azo groups.

1.6. Classified Dyes According to Dyeing Property

Based on the dyeing properties dyes are classified as acid, basic, direct, reactive, disperse, mordant, and metal complex dyes. Reactive dyes are commonly used in the textile industry. These dyes are the only group of dyes that can establish covalent bonds with the fiber under optimal conditions. Reactive dyes are mostly used for preparing blue, red, and yellow colors. The acid dyes contain functional groups of sulfonic or carboxylic acid salt that provide diffusion and migration of dye molecules on the positive surfaces. Direct dyes can enter the cellulose fibers having good affinity. Mordant dyes are acid dyes with specific regions of acid salt anion group groups that can react with a

metal salt mordant. Mordant dyes couple with metal ions to form a strong organometallic complex with solubility and higher colorfastness. Basic dyes have the cationic character which is colored cationic salts of amine derivatives. Basic dye cations will migrate toward negative charges inside the fiber. Disperse dyes were formulated to dye hydrophobic thermoplastic fibers. The dispersed dyes are small polar molecules, usually containing anthraquinone or azo groups, which do not have cationic or anionic charged groups in their structure.

2. EFFECTS OF DYESTUFF ON ENVIRONMENT AND HEALTH

Industries, especially textile industries, which use synthetic dyes, produce a high amount of dye contained wastewater. About 7 x 105 tons of commercial dyes and pigments are produced per year worldwide (Sponza et al. 2000; Kaykıoğlu and Debik 2006). About 10- 15% of the dye is discharged into the receiving water (Gomez et al. 2007). Approximately 40-65L of wastewater is produced per one kg of product in the textile industry (Manu and Chaudhari 2002). Each year about 280000 tons of dye contained wastewater is discharged to the receiving environment all over the world (Maas and Chaudhari 2005). Dyes are the most obvious signal of water pollution and some dyes are visible even at very low concentrations as 0.005 mg/L (O'Neill et al. 1999). The range of dye content of the textile industry wastewater is 10- 200mg/L (O'Neill *et al.* 1999). Therefore usually highly colored wastewater is discharged into open waters and caused

aesthetic problems. A large part of commercially used synthetic dyes is azo dyes that have toxic, mutagenic, and carcinogenic properties (Seesuriyachan et al. 2007). About 15% of these toxic dyes are dispersed into the environment after being used for operations such as washing (Carliell et al. 1995; Philips 1996; Swamy 1998). If the dye contained textile wastewaters aren't treated, they affect the environment and human health negatively.

The majority of dyes pose a potential health hazard to all forms of life. Dyeing wastewater reduces light transmission and affects the photosynthetic activity of aquatic life negatively (Aksu 2005). These dyes also have a toxic effect on living organisms. For example, some toxic molecules are produced after the digestion of azo dyes in the mammal body (Rafii et al. 1995). Dye contained wastewater reached the human body cause a toxic effect on body systems (Özcan ve Özcan 2004). Table 1 shows the effect of the dye contained wastewater on the human body.

Table 1: The effects of textile dye on human health

Effect	Reference
Allergic reactions	Sivarajasekar & Baskar 2014
Irritation to the skin and respiratory tract	Mani & Bharagava 2016
Renal failure	Mani & Bharagava 2016
Potential mutagenicity	Chequer et al. 2009
Disorders of the central nervous system	Khan & Malik 2018
Dermatitis	Khan & Malik 2018
Carcinogenicity	Lacasse & Baumann 2012
Cytotoxicity	Fernandes et al. 2015
Genotoxicity	Thakur 2006
Asthma	Hassaan 2016
Rhinitis	Hunger 2007

Particularly, dyes used in the textile industry are reported as toxic, genotoxic, and mutagenic effects in test systems. Dyes having azo bonds are carcinogenic and cause tumors of the liver and urinary bladder in experimental animals (Lacasse & Baumann 2012). The aromatic amines and several aromatic amines found in dye structures are known as mutagens and carcinogens. The toxicity of aromatic amines is related to the nature and location of other substituents. For instance, the substitution with nitro, methyl, or methoxy groups or halogen atoms may increase the toxicity (Chung and Cerniglia 1992).

3. DECOLORIZATION OF DYES VIA THE BIOLOGICAL MATERIALS

As mentioned before, dyes released into aquatic environments through textile wastewater cause important environmental and health problems

for the living creatures in these environments. Therefore, the wastewater containing textile dyes should be treated before being released into the environment (Gül 2020). Today, biological treatment methods, which are economical and environmentally friendly, have gained importance in green technologies. Decolorization of textile dyes by biological methods involves three mechanisms called biosorption, bioaccumulation, and biodegradation (Fu and Viraraghavan 2001).

Biosorption is defined as an effective technology using inactive and dead biomasses to remove pollutants from aqueous solutions. Bioaccumulation is the take of pollutants by growing cells. Biodegradation involves the degradation of pollutants into various chemicals using enzymes. From the past to date, most of the researches shows that all of these three mechanisms have been used by biological systems and successful treatment results have been gained (Chu & Chen2002; Gül 2018; Gül 2020).

CONCLUSION

Synthetic dyes are extensively utilized in the textile industry. Therefore, textile wastewater includes large amounts of dyes that come out as a result of the dyeing process. This study aims to review the structure of textile dyes and their effects on the environment and health. According to the results of this study, synthetic dye's presence at high amounts in effluents of the textile industry have negative effects on the environment and health. Due to the pollution problems related to textile dyes, the usage of dyes is under constant

development, and also the attention increased being paid to the harmful effects of these chemicals. Today, scientists and governments deal with environmental and public health-related to the discharge of dyeing effluents from the industries and, also wastewater treatment systems. In this context, scientific studies suggesting new and innovative methods for the treatment of industrial wastewater are still important.

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BÖLÜM 2

MOLECULARLY IMPRINTED POLYMERS IN BIOSENSOR TECHNOLOGY

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1. INTRODUCTION

1.1. Introduction the Polymers

Polymers are large molecular weight molecules formed by monomers, a single molecule connected by covalent bonds. The reactions in which monomers bond with chemical bonds to form polymer molecules are called polymerization reactions. The number of repeating units in the polymer molecule is called the polymerization degree (Pn). Polymerization degree is one of the parameters that determine the properties of polymers. Polymers can consist of a single type or more than one type of monomer. Polymers consisting of a single type of monomer are called homopolymer, and polymers that consist of more than one type of monomer are called copolymers(Allen & Bevington, 1989).

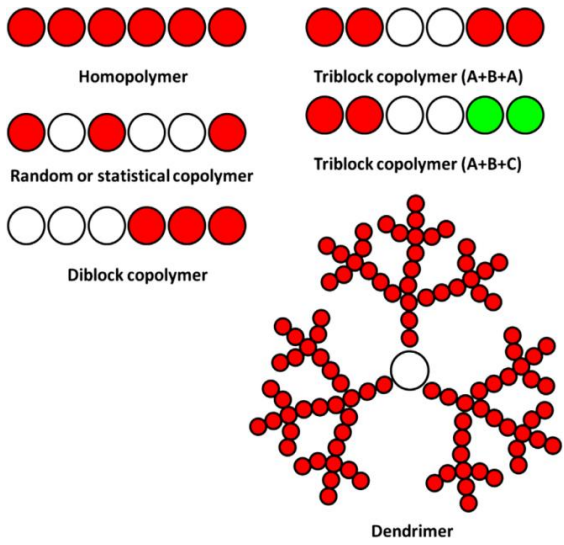


Figure 1. A linear homopolymer, a diblock, an A+ B+A and A+B+ C triblock copolymer, a random or statistical copolymer, and a dendrimer are all examples of block copolymers (Bansal et al., 2017)

1.2. Basic Properties of Polymers

The most essential feature of polymers is the glass transition temperature (Tg). Since polymers have large molecular weights and consist of chains with different molecular weights, a precise melting point cannot generally be mentioned. The usage properties and areas of polymers can be determined by using the glass transition temperature. The polymers are hard and brittle below the glass transition temperature, and above it, they are rubbery and soft.

In addition to the polymers obtained synthetically under laboratory conditions, there are also natural polymers in nature. Examples of natural polymers are starch, cellulose, enzyme, deoxyribonucleic acid (DNA), cotton, wool, natural rubber, and similar biological origin macromolecules. Since these natural substances have substantial molecular weights, they show different and superior properties. Rapidly developing technology and increasing competition have led to the development of the production of more superior and qualified synthetic polymers. Properties such as high elasticity, viscosity, resistance to heat and corrosion, and easy shaping caused polymers to be used frequently in the industry (Billmeyer, 1984).

1.3. Polymerization Techniques

Since humanity existed, polymers have been used for many years, but the word "polymer" was unknown. After the 19th Century, polymers came into daily life. In 1984, the production of polymer fibers was equal to the cotton fabrication. Moreover, nowadays, polymers, especially synthetic ones, are everywhere(Seymour, 1989). In their

production for biosensor technologies, polymers are used for components for enhancing selectivity, ease of operation, and high affinity. In recent studies for biosensors -especially for molecularly imprinted polymers- different types of polymerization techniques are used. Some of the most used techniques are bulk polymerization, electropolymerization, and precipitation polymerization. Recovery of the material is important for biosensors for reusing. Bulk polymerization has good recovery (Abdullah Youssef, 2019). Also, with the electrical polymerization method, layer thickness and morphology can be controlled very well. These two properties affect the biosensor performance like paper-based biosensors (Crapnell et al., 2019). Different types of polymerization techniques are used for different biosensor applications, and those different types of polymerization techniques may affect biosensor's selectivity, stability, affinity, reusability, and also cost, which is another crucial parameter for molecularly imprinted polymer-based biosensors. In this part, Bulk, Electro, Emulsion, Precipitation, and Free Radical Polymerization methods are investigated.

1.3.1. Bulk Polymerization

Bulk (or mass) polymerization is a solventless process, and also dispersants are not used. The process is simple and very common among other polymerization techniques. Especially for MIP production, bulk polymerization is preferred. Operation costs are very low, and since there is no solvent, environmental risks are reduced, such as contamination and emission (Tartamella, Cole, & Smale,

2008). In bulk polymerization, monomers, initiators, and chain transfer agents are included in the reaction mixture (Rudin & Choi, 2013). Chain transfer agent controls the molecular weight. Heat is important for this process to grow polymers. Heat is given to the mixture, and the mixture is continuously agitated. When the reaction starts, the heating process is also stopped, and after some time, viscosity increases because polymerization occurs, so the agitation rate must be controlled very carefully. Polymerization reactions are not very exothermic, so high amounts of heat are needed, but equipment for this process has a very low cost (Abdullah Youssef, 2019).

1.3.2. Electropolymerization

Electropolymerization is an electrosynthesis method for fabricating polymers. It is a deposition method. As a result, a conductive polymer layer or a coated polymer can form on an electrode (Crapnell et al., 2019). The working principle is similar to metal coating. Three electrode systems (reference electrode, working electrode, and counter electrode) are used with a solution. This solution includes monomers, solvent, and the electrolyte. Potential and current are applied to the system and has to be carefully controlled. The optimal potential value is needed for monomers' oxidation process, and the high potential means dissolution of metal, and it is undesirable for the polymerization process. After the oxidation process, the polymerization process of monomers occurs. The process is conducted in an electrochemical cell. There are different types of this method.

In galvanostatic electropolymerization, the current is kept constant, and polymerization occurs at the same rate. For potentiodynamic electropolymerization, there is a cyclic and regular potential change to stabilize a polymer film growth. In potentiostatic polymerization, the potential is constant. This constant potential controls the polymerization rate. Electropolymerization is applied when small areas like biosensors come to play. The polymer film can be obtained with controllable thickness and rate with desired templates and morphology. The final product depends on applied potential, current, solvent, electrolyte, and monomer type. It is a suitable method for molecularly imprinted biosensors when a polymer layer is needed for the application (de Leon & Advincula, 2015).

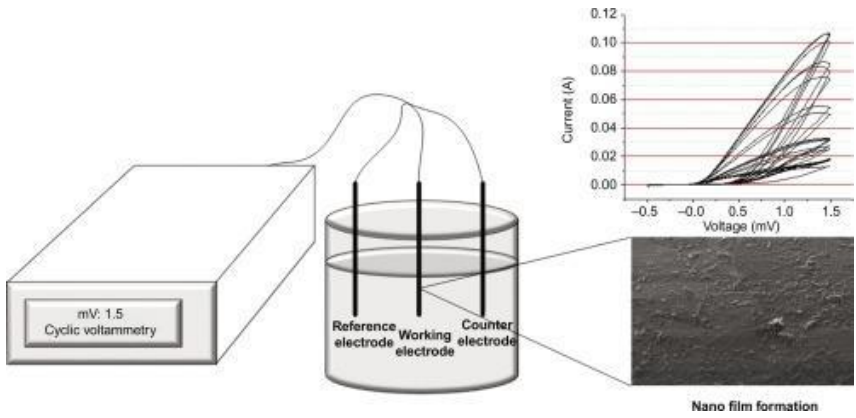


Figure 2. Electropolymerization schematics of dopamine on a nitinol substrate (Aguilar, 2019).

1.3.3. Precipitation Polymerization

Precipitation polymerization is a simple method. There are no stabilizers for the polymerization reactions. Uniform, homogeneous particle size is obtained in this process. The first step is forming oligomers from monomers. Then cross-linking of oligomers occurs. After these steps, particles start to grow. New polymers are precipitated from the solution. To obtain uniform and homogeneous particles, precipitation speed, monomer, and cross-linker choices must be controlled. The homogeneity of the particles determines the performance of the final application. If there is no homogeneity, performance decreases. If an application needs the right and uniform particle sizes such as microspheres and clean polymers, precipitation polymerization is a suitable method (Li, Möhwald, & Shchukin, 2013).

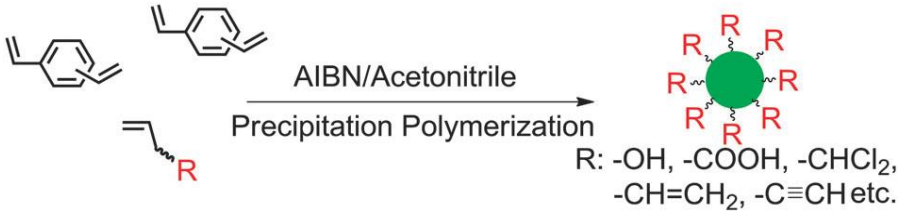


Figure 3. Functional polymer microspheres by precipitation co-polymerization of vinyl monomers with a di-functional with a di-functional cross-linker (Li et al., 2013).

1.3.4. Emulsion Polymerization

Emulsion polymerization is a good method for obtaining high surface area with small particles. 2D layers can be obtained by using emulsion polymerization. An organic phase that contains functional monomer,

cross-linking agent, surfactant, and the initiator and an aqueous phase are prepared. Ultrasonication of organic phase occurs. The organic phase and aqueous phase are mixed together. After the mixing, a homogenizer agitates the mix. With agitation, polymerization occurs. Finally, the cleaning process is done, and the polymer is obtained. The production time is short, and high molecular weight polymers can be formed. The polymer can be cleaned easily (Özkütük et al., 2016). For biosensor applications, if reproducibility is essential, emulsion polymerization would be the right choice. Elution of the molecularly imprinted polymers is done easily, and biosensors can be used many times after the use (Belmont et al., 2007).

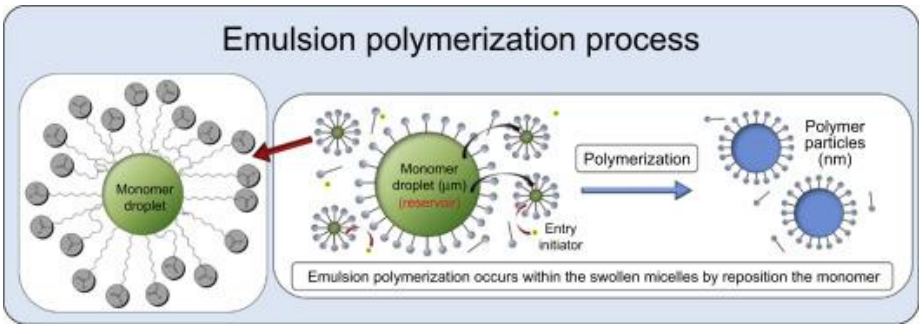


Figure 4. The radical initiators and their entrance mechanism are depicted in this diagram of a conventional emulsion polymerization process. Diffusion of monomers into micelles and the micellar structure are depicted as a self-organized process including monomer droplets (head as the hydrophilic part and tail as the hydrophobic part) with a surfactant concentration greater than the critical micelle concentration (Jensen et al., 2017).

1.3.5. Free Radical Polymerization

Free Radical Polymerization is generally used for producing hydrogels. For gel applications, especially in biotechnology, free radical polymerization offers good operation conditions and properties. Polymerization occurs in a short time. There are several steps for polymerization. These are 1) Initiation, 2) Propagation, 3) Termination. In the initiation step, initiators react with monomers and create free radicals. These initiators can be redox agents, thermal or visible lights. In the propagation step, formed free radicals react with monomers. With propagation, polymer chains are started to form. In the termination step, chains are bonded together. Radicals are removed from the chains, and these chains are bonded with covalent bonds. And then, the polymer is formed (Varghese, Rangappa, Siengchin, & Parameswaranpillai, 2020).

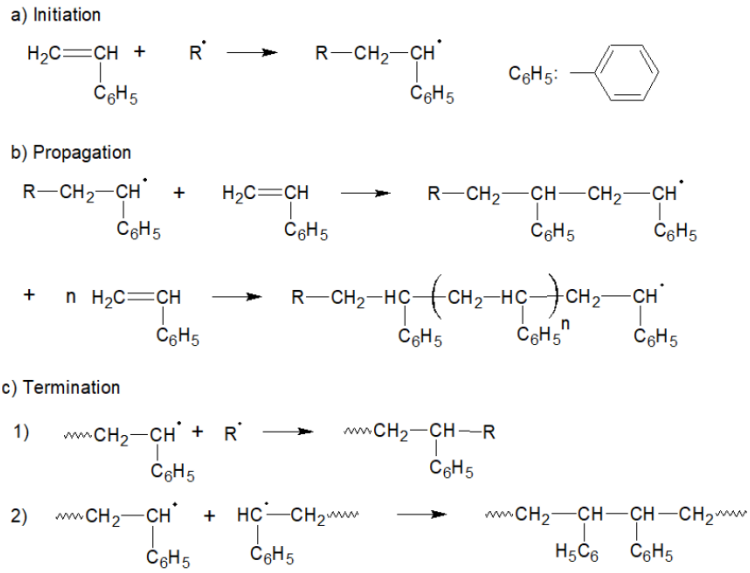


Figure 5. Free radical polymerization of Polystyrene ("Polystyrene," n.d.)

1.4. Usage areas of Polymers

Both natural and synthetic polymers are notably involved in the comfort and facilitation of human life, and information technologies, medicine, nutrition, communications, transportation, irrigation, containers, clothing, registration history, buildings, highways, etc. areas directly responsible for life itself. The imagination of human society is difficult without synthetic and natural polymers. Polymers can primarily be produced at low costs. Their light and workable structures allow them to be used in many different sectors. Polymers are gradually replacing metals in many high-tech areas, from the space industry to the aircraft industry. Due to their high resistance to heat changes, they can find a place in the food sector ("Kapalı Polimer Kanal Fiyatları," n.d.).

1.5. Biosensor Systems

While clinical electrochemistry may be used to make sensors that just detect anions and cations, biomaterial can be added to the system to detect a variety of different chemical and biological molecules. As a result, the created analytical systems are referred to as biosensors (Aykut & Temiz, 2006). Biosensors are analytical devices that contain biological diagnostic material that will enable the identification of analytes and convert the product formed as a result of the interaction between the analyte and the biological diagnostic material into a measurable signal with the help of a physicochemical converter (Paddle, 1996). In1962, Leland C. Clark Jr. reported enzyme immobilization at electrochemical detectors. With this research,

enzyme electrodes are obtained, and these enzyme electrodes increase the range of the analyte based sensor. These ideas are considered as the beginning of the modern concept of biosensors (Turner, Karube, & Wilson, 1987). Biosensors are analytical devices that convert a biological response into an electrical signal. In essence, biosensors must be highly specific and reusable, independent of physical parameters such as pH and temperature. The materials used in biosensors are divided into three groups according to their mechanism: a biocatalytic group consisting of enzymes, a bioaffinity group containing antibodies and nucleic acids, and a microbe-based containing microorganisms (Thévenot, Toth, Durst, & Wilson, 2001).

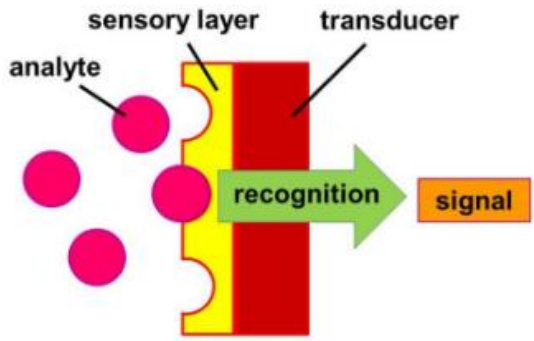


Figure 6. Simple shape of the biosensor structure("Optical Biosensors in Medical Diagnostics," n.d.)

1.5.1. Working Principles of Biosensors

The biosensor system consists of three essential components. These are the biomolecule with selective recognition mechanism, the "converter" and "electronic" parts that convert the physicochemical

signals generated due to the interaction of this biomolecule with the substance under investigation into electronic signals. The most important of these components are highly-selective but reversibly sensitive biomolecules against the substance to be determined. As a biomolecule in biosensors; Enzymes, microorganisms, organelles, tissue sections, antibodies, nucleic acids, and chemical receptors embedded in biological membranes can be used. Biomolecules involved in the structure of biosensors are often referred to as receptors. Bioeceptors transform the substance to be analyzed, and the changes accompanying this transformation are detected by the signal converter. The most commonly used bioreceptors are enzymes due to their high specificity. Cell systems or microorganisms are used when a suitable enzyme cannot be found, or the enzyme is unstable, and multiple substances are determined. In the presence of the substance under investigation, the biomolecule should have a physicochemical effect that can be determined by a transducer (Vo-Dinh & Cullum, 2000).

Transducers can detect electrical signals via converting chemical energy to electrical energy or detect different analytes' wavelengths like colorimetric detection. They convert bioactivity to a signal that can be monitored. Transducers have different types such as electric potential, electric current, intensity, mass, temperature, and so on. Amplifiers can also be used with transducers to increase and clarify the signal (Mohanty & Kougianos, 2006).

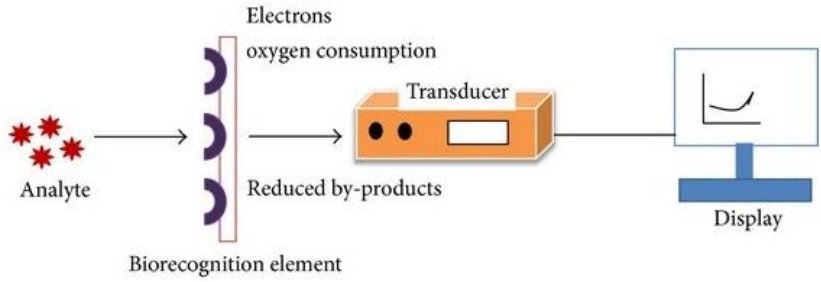


Figure 7. Working principles of a Biosensor (Dhull, Gahlaut, Dilbaghi, & Hooda, 2013)

1.5.2. Application Areas of Biosensors

Biosensors play a very important role in medicine, agriculture, food, pharmacy, environmental pollution, defense, and many industrial areas, especially in automation, quality control, and energy storage. Biosensors can be used to determine organic substances such as foods, metabolites, antibiotics, drugs, some inorganic compounds, and enzymes, viruses, and microorganisms. Undoubtedly, the biomedical sector is the best market for biosensors. Enzyme sensors are the first biosensors to find application in this field. The first commercially produced biosensor is the glucose oxidase electrode that enables glucose determination in blood and urine to diagnose diabetes (Azmi Telefoncu, 1999).

1.6. Polymers in Biosensor Systems

Polymers have extensive usage for energy applications, daily lives, and medical purposes. New conductive polymers are not only used for biosensor technologies but also used for lithium batteries. New drug delivery systems use new polymeric materials. However, for

biosensors, polymers became indivisible from biosensor systems. New wearable techniques use polymers as a primary material because of their flexibility. On the other hand, conducting polymers offer good immobilization properties (Teles & Fonseca, 2008).

Microfluidic channels and polymer arrays are used for biosensor technologies. One polymer type is very attractive: Polydimethylsiloxane. It is flexible and lightweight, and also the production of PDMS has a low cost. Koh and coworkers reported a microfluidic device that can be worn, and this device can measure the sweat rate, pH, glucose, and lactate concentration. This device's main structures are cover polydimethylsiloxane (PDMS) and PDMS microfluidic channels. It is a colorimetric biosensor. The sweat sample moves to the different parts of the biosensor, and with the change of color intensity, measurements are performed. PDMS has good properties like optical transparency, easy patterning methods, flexibility, and biocompatibility (Koh et al., 2016).

Conducting polymers are also the most used polymers in biosensors, especially in enzyme immobilization. Soganci and coworkers have reported an electrochemical biosensor that uses conducting polymers as an immobilization matrix. In this study, they synthesized the BTP with a better structure to improve electrical conductivity, optical properties, and stability. This conducting polymer also increases the performance and selectivity of the biosensor (Soganci, Soyleyici, Demirkol, Ak, & Timur, 2018). Azak and coworkers have also reported a new glucose biosensor with conducting polymers. They

used DTP-Ph-NH2 and DTP-Ph-Ph-NH2 type monomers as immobilization matrices. After the tests, they revealed that sensitivity and selectivity are increased. These conducting polymers decrease the time of the electron transfer and immobilization. Their consistency with analytes and good electrochemical properties makes them a suitable choice for the immobilization matrices (Azak, Kurbanoglu, Yildiz, & Ozkan, 2016).

2. MOLECULARLY IMPRINTED POLYMERS (MIP)

Molecularly Imprinted Polymers (MIPs) are robust molecular recognition components capable of mimicking natural recognition entities such as antibodies and biological receptors that are useful for distinguishing and analyzing complex samples such as biological fluids and environmental samples obtained using imprinting technology. The process of molecular printing is utilized to construct artificial recognition sites in polymeric matrices. In terms of size, form, and spatial arrangement of functional groups, these synthetic recognition sites should be complimentary to the template. Covalent, non-covalent, and semi-covalent interactions can be used to achieve these spatial configurations between the template molecule and functional monomers. The cross-linking monomers and functional monomers that constitute the pre-complex and the template molecule are then co-polymerized. The template molecule is removed after polymerization, leaving complementary gaps that can be used as fake recognition sites (Vasapollo et al., 2011).

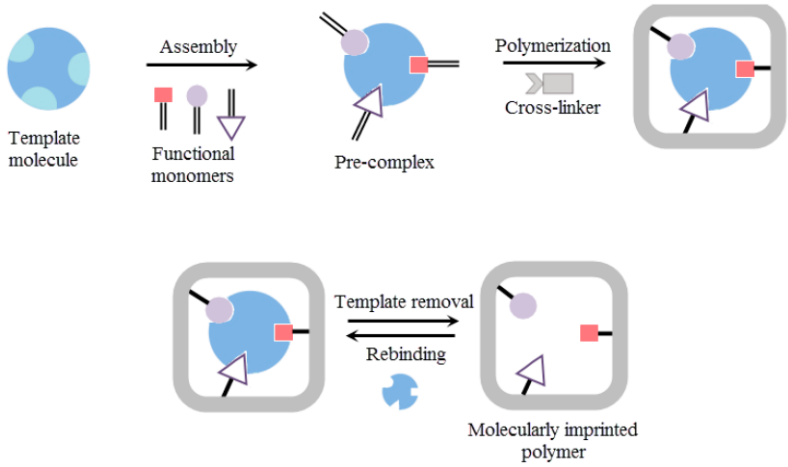


Figure 8. Scheme of the principle of molecularly imprinted polymer preparation (Saylan, Akgönüllü, Yavuz, Ünal, & Denizli, 2019).

2.1. Classification of MIP Biosensors Based on Their Transducers

Biosensors can be classified as bioactive layers and transducer types. It is possible to categorize as more stable synthetic structures as well as types of bioactive layers and MIPs based on biomolecules such as enzymes, antibodies, receptors, and nucleic acids (aptamer, DNA, etc.). If it is necessary to classify it in terms of the most commonly used transducers (Vasapollo et al., 2011), we can list them as electrochemical, optical, mass sensitive transducers (Gaudin, 2017).

2.1.1. Electrochemical MIP Biosensors

Electrochemical biosensors measure the electrical changes of the solution, such as the electric potential or current, caused by the production or consumption of electrons or ions. In electrochemical MIP biosensors, these changes occur when the target molecule

chemically interacts with certain MIP cavities, the biosensor's bioactive layer. An electrochemical sensor is a device that converts the interaction of an analyte with a receptor on the surface of an electrode into a useful analytical signal (Hulanicki, Glab, & Ingman, 1991). Potentiometry, voltammetry, conductometry, and impedimetry are techniques used to convert chemical interactions into electrical signals.

Another trend in the development of electrochemical MIP sensors is to use microparticles and nanoparticles (NPs) or nano-structured coatings.

By accelerating electrochemical processes, gold NPs (AuNPs) enhance the electrode surface, improve the electron transfer process between electroactive species, and raise the sensitivity of electrochemical sensors, drawing attention as an appealing technique. The voltammetric MIP sensor for adenine based on AuNPs covered with 3-thiophene acetic acid, which results in a conductive 3D mesh composite film after polymerization, is another example of nano-engineering (Figure 9). With a 0.99 nM LOD, this form of sensor construction has increased selectivity and sensitivity for adenine detection (detection limit) (Wang, Zheng, & Xu, 2018).

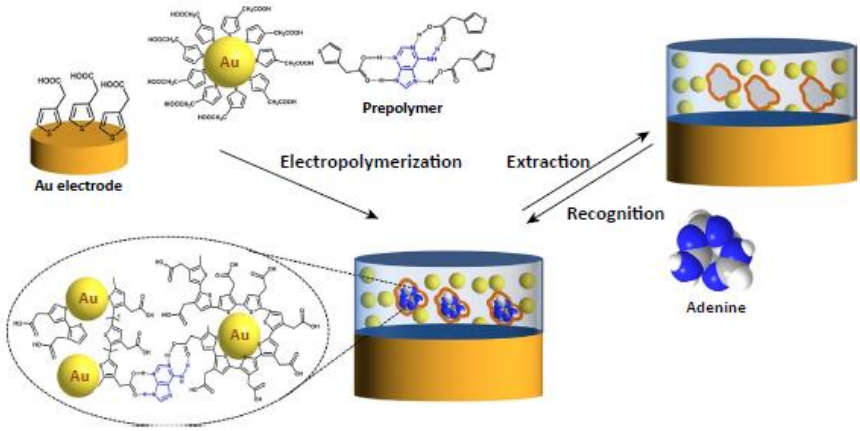


Figure 9. Fabrication of an Adenine Voltammetric Sensor Using a Conductive 3D Network Composite Made of Molecularly Imprinted Polymer (MIP) (Ahmad, Bedwell, Esen, Garcia-Cruz, & Piletsky, 2019)

2.1.2. Optical MIP Biosensors

Optical sensors use light to convert chemical interaction into measurable signals. If the system's optical properties change with the binding of the specific analyte of the MIP, which is the biosensor's bioactive layer, the analyte can be analyzed with the biosensor with an optical transducer. Optical-based assay techniques consist of several subclasses based on different optical principles such as SPR, RLS, fluorescence, luminescence, and Raman spectroscopy (Vasapollo et al., 2011).

SPR Based MIP Biosensors: Surface plasmon resonance (SPR) is an optical technique for measuring the refractive index changes at the surface of a metal to characterize affinity interactions between biomolecules. SPR-based biosensors have the potential to be used in

bioscience, food analysis, and environmental analysis due to their unique properties such as real-time measurement, high sensitivity, specificity, reproducibility, and no need for labeling (Ertürk, Uzun, Tümer, Say, & Denizli, 2011). Optical fiber surface plasmon or localized surface plasmon resonance sensor also provides highly accurate real-time sensing with fast response times, ease of use, online monitoring, and remote sensing (Gupta, Shrivastav, & Usha, 2016). In addition, molecular imprinted polymers with selective binding sites for the target molecule in SPR sensors have some advantages: easy preparation, low cost, and stability (Dibekkaya, Saylan, Yılmaz, Derazshamshir, & Denizli, 2016).

Figure 14 shows the mechanism of the SPR chip, which was performed by Shaikh et al. 2015 (Shaikh et al., 2015). They suggested a study for the development of an SPR sensor with synthesized Bisphenol A (BPA) imprinted poly(ethylene glycol dimethacrylate-N-methacryloyl-L phenylalaninevinyl imidazole) [poly(EGDMA-MAPA-VI)] film via radical polymerization under UV light. Characterization of the SPR sensor was performed by FTIR-ATR, atomic force microscope, and ellipsometry. SPR sensor developed to detect BPA in Milli Q water, tap water, and synthetic wastewater was used. The concentration range of detection of BPA was 0.08 to 10 mg / L. The LOD and LOQ values of the sensor were 0.02 and 0.08 mg / L in Milli Q water, 0.06 and 0.2 mg / L in tap water, and 0.08 and 0.3 mg / L in synthetic wastewater. The adsorption system is suitable for the Langmuir adsorption model. Selectivity studies were carried out with competitive detection of 4-nitrophenol, hydroquinone, phenol,

and 8-hydroxy quinoline and showed that the molecularly imprinted film preferred BPA over 4-nitrophenol, hydroquinone, phenol, and 8-hydroxy quinoline with a relative selectivity coefficient of 2.5, 2.6, 2.7 and 2.5, respectively.

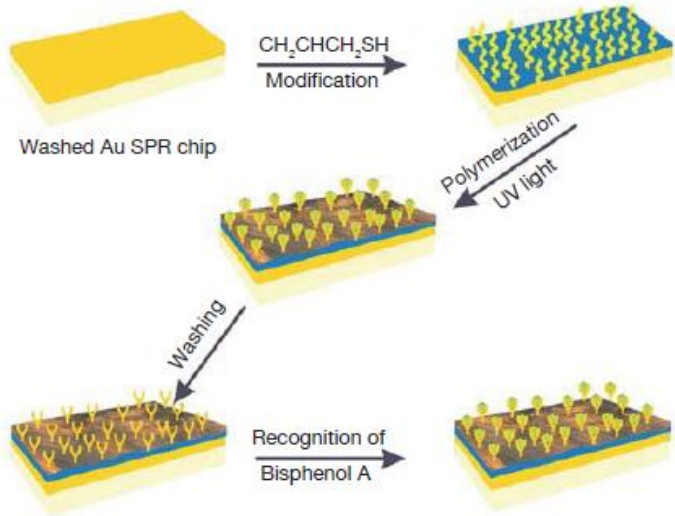


Figure 10. Preparation steps for the BPA imprinted SPR sensor chip (Shaikh et al., 2015).

Fluorescent Based MIP Biosensor: Fluorescence detection; has advantages such as high sensitivity, comfort, and wide application range. MIP-based fluorescence sensors combine the advantages of the high selectivity of MIP recognition and high sensitivity of fluorescence detection. MIP-based fluorescence detection can be used directly and indirectly. For direct fluorescence detection, the analyte is active to alter the signals for quantitative and qualitative analysis due to changes in the fluorescence intensity of the MIPs. For indirect

fluorescence detection, non-fluorescent analytes are used for detection, and the fluorescence effect is obtained from the fluorescent functional monomer by measuring the change in the fluorescence spectrum of the combination of analytes with a fluorescent functional monomer (L. Chen, Wang, Lu, Wu, & Li, 2016).

Xu et al. reported a study that is a mesoporous structured MIPs@CDs fluorescence sensor for highly sensitive detection of trinitrotoluene (TNT). TNT is important for particularly global safety, and it is toxic to all creatures. Thus, detection of TNT in a selective and extremely sensitive way is quite essential. For this aim, mesoporous structured molecularly imprinted polymers capped carbon dots (M-MIPs@CDs) were prepared. All the steps were shown below in figure 15. Amino-CDs were functional monomer and trinitrophenol (TNP) as dummy patterns for imprinting. Characterization studies were transferred out by fluorescence measurements, TEM, and SEM microscopy. M-MIPs@CDs sensor has a detection limit of 17 nM. The sensor was stable 10 times without yield decrease. The improved sensor was realized in soil and water samples with 88.6-95.7% recoveries (Xu & Lu, 2016).

It was improved molecularly imprinted fluorescent empty nanoparticles as sensors for rapid and efficient detection λ-cyhalothrin in environmental water (Xu & Lu, 2015). λ-cyhalothrin (LC) is a toxic insecticide. In order to detect λ-cyhalothrin quickly and efficiently in water samples, SiO2 nanoparticles were synthesized with a Stöver method. Then they were modified with fluorescein isothiocyanate

(FITC). LC imprinted fluorescent empty nanoparticles were prepared. Thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), TEM, and SEM. Detecting fluorescence density range is 0-1000. The detection limit was 10.26 nM, and the fast detection rate was 8 min. 8 cycling operation was obtained with good fluorescence properties. This improved sensor was used to detect λ-cyhalothrin in real water samples from Beijing-Hangzhou Grand Canal Water with good recovery.

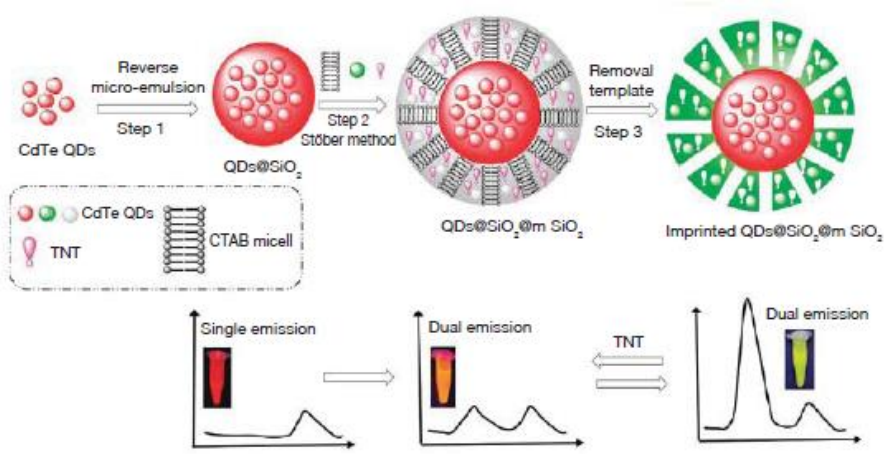


Figure 11. TNT sensing techniques and the manufacture of mesoporous structured MIP@CDs ratiometric fluorescent probes are shown schematically (Xu & Lu, 2015).

2.1.3. Mass Sensitive MIP Biosensors

Mass sensitive transducers, also called gravimetric biosensors, detect the mass of analyte in solution over time. They work by generating and sensing acoustic or mechanical waves. Piezoelectric crystals, quartz crystal microbalances (QCM), and surface acoustic waves (SAW) are the most common components of mass sensitive

transducers. The pressure created by the accumulation of some solid materials such as ceramics, especially crystals, or some organic materials such as protein, DNA and bone also can cause mass changes in the region due to this pressure. The electric charge that detects this accumulation and causes mass changes is called a piezoelectric. In acoustic wave sensors, waves propagate on the surface of the piezoelectric material. In mechanical wave sensors, bend and mass changes are detected by the optical phenomenon. The Quartz Crystal Microbalance (QCM) is also popular among mass sensitive biosensors (Vo-Dinh & Cullum, 2000).

Figure 16 shows a study by Naklua et al. get. In 2016, QCM sensors based on molecularly printed polymers were reported to detect the dopaminergic receptor (Naklua, Suedee, & Lieberzeit, 2016). Agonist and antagonist dopamine D1 receptor (D1R) were measured with the QCM sensor. The template was D1R derived from the rat hypothalamus. Acrylic acid: N-vinylpyrrolidone: An oligomer film of N, N '(1,2-dihydroxyethylene) bis-acrylamide was pressed onto a dual-electrode QCM. Characterization of successful D1R print has been verified by AFM. LoD was 4.3 μM and LoQ was 5.9 μM. The concentration range was 5.9-47.2 μM. D1R-MIP has proven to be highly beneficial for selectively binding protein. This is the theoretical value because the protein is bound to cell surfaces. Besides, investigation of free receptor and receptor-dopamine complexes has proven useful.

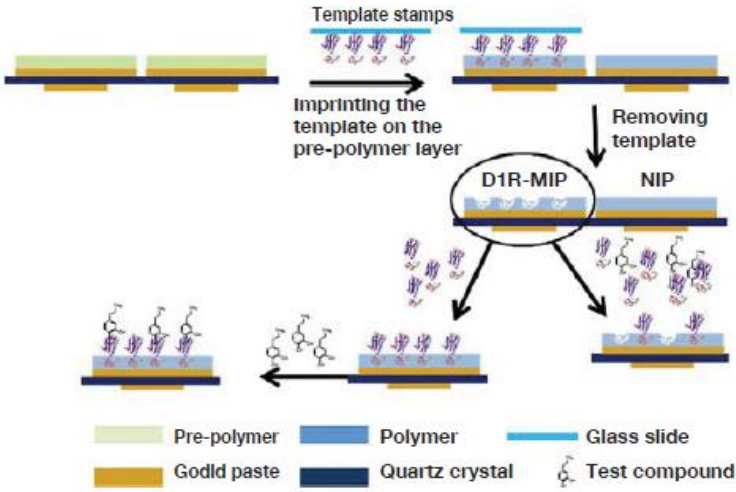


Figure 12. Schematic synthesis strategy for D1R-MIP and NIP on QCM electrode (Naklua et al., 2016)

2.2. Advantages of MIPs

MIPs offer many challenging advantages. Compared to enzymes or antibodies, they can be produced very easily, and the manufacturing process is very cost-effective. Regeneration after usage is favorable, and they show both storage and operational stability. In particular, they show selectivity and binding strength. Because of their high sensitivity and selectivity, MIPs are considered plastic antibodies or plastic bodies and show even better results compared to commercial antibodies. Another striking point of MIPs is the ability to modify the entire polymer structure or just the functional regions of the MIP, thus using different monomer / functional monomers in different ratios. Therefore, MIPs can have excellent stability even when exposed to

extreme pH, organic media, or autoclave conditions (Mattiasson & Ertürk, 2017).

Moreover, the story of MIPs, which started with block polymerization, have mostly transformed into the story of smaller particles nowadays. They can be synthesized as micro / nanoparticles with higher specific surface areas, giving them favorable binding properties. MIPs are used in many applications in purification, isolation, biosensors, catalysis, and chiral separation, with all their advantages (Ertürk & Mattiasson, 2017).

2.3. Importance and Usability of MIP Biosensors

If we need to mention the biosensor usage of MIPs, one common method is the formation of MIPs directly on the electrode surface. In addition to this MIP, micro/nanoparticles can be synthesized separately and then immobilized on the electrode surface by co-polymerization or combining various interactions. In particularly, Nano-sized MIPs can compete with antibodies when successfully designed and synthesized. For this, the stability of the MIP is an important factor provided by the amount of cross-links in the polymer structure. Besides, MIP-based sensors are often described as "biosensors," even though MIP is not actually a biological part. One of the applications of MIPs in analytical processes is their use as solid-phase extraction supports. Here, the MIPs are treated with a medium containing the analyte, and then the MIPs are washed to obtain a concentrated fraction for analysis. Also, the surface printing method

can be used to obtain different MIP biosensors (Mattiasson & Ertürk, 2017).

3. Preparation Techniques of MIP based Biosensors

3.1. Essential points in Molecular Imprinted Polymer Preparation Techniques

The molecular imprinted polymer preparation method generally consists of three steps. First, binding is performed between the functional monomers and the template molecule. Then the functional monomer-template complex formed by the cross-linking chemical is polymerized. Finally, the template molecule is removed from the polymer with a suitable washing chemical. Two different approaches are used to prepare molecular imprinted polymers based on the interaction between the template molecule and functional monomers. One of these is Wulff et al. It is a pre-organization method developed by (1977). In this method, the template molecule and functional monomers are linked to each other by covalent bonding, and this complex structure maintains its stability throughout polymerization (Wulff, Grobe-Einsler, Vesper, & Sarhan, 1977). Another approach Mosbach et al. (1981) instead of covalent bonding is a non-covalent approach that includes interactions such as hydrogen bonds, hydrophobic interactions, van der Waals interactions, and Coulomb interactions between ionic groups (Arshady & Mosbach, 1981).

In molecular imprinting techniques, binding sites of the complex are very important. The target can be bonded with these binding sites selectively, and detection can be done precisely and accurately. Reusability and reproducibility are also important parameters for MIP, and interactions between target and binding sites affect these parameters. These interactions can be classified as covalent and non-covalent. Non-covalent interactions also can be classified as van der Waals, hydrogen, metal coordination, or ionic. Covalent bonds are strong bonds compared to non-covalent bonds, but this can be a problem because breaking the bonds for reusing can be difficult. The reversible reactions are limited when covalent bonds are in the system. Also, slower binding and removing makes the process undesirable. However, the selectivity will be high due to its strong binding capacity. For non-covalent interactions, reversible reactions are favorable, but selectivity can be decreased due to the weak bonds. To overcome this problem, a high amount of monomers can be used in a MIP, but this can lead to heterogeneous binding sites. Heterogeneity can decrease affinity. In a complex system, there can be different types of interactions. For obtaining better properties for MIP, new methods are studied. One of them is semi-covalent interaction. In this interaction, bonding between the template and binding site acts as a covalent bond, and rebinding acts as non-covalent interaction (L. Chen et al., 2016).

Table 1. Chemical and Physical Interactions

Interaction	**Bond Type**	**Interaction Type**
Covalent	Strong Covalent Bonds	Chemical Interaction
Non-covalent	Van der Waals Hydrogen Metal Coordination Ionic	Chemical or Physical Interaction
Semi-Covalent	Intermediate Bond Between Non-covalent and Covalent	Chemical and Physical Interaction

Chen and coworkers reported a MIP nanoparticle for detecting lysozyme. They used metal coordination interaction between template and target. They observed high rebinding capacity and rapid binding kinetics. Metal coordination interaction provided a strong affinity and selectivity (H. Chen, Kong, Yuan, & Fu, 2014).

Caro and coworkers reported a MIP for extraction of 4-nitrophenol from water samples. They used non-covalent interactions and semi-covalent interactions. High selectivity is observed in the non-covalent MIP, but higher recoveries are observed in the semi-covalent MIP (Caro et al., 2002).

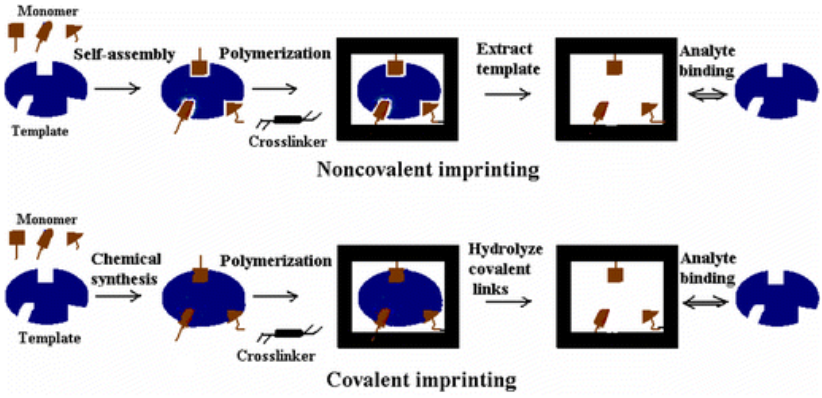


Figure 13. Schematic Representation of the Covalent and Noncovalent Molecular Imprinting Procedures (Qiao, Sun, Yan, & Row, 2006)

Free radical polymerization is the most used technique in the preparation of MIPs, as it is an economical method besides its versatile applications and experimental convenience. Today, this technique is the most preferred in the industry. Polymerization can be initiated by thermal degradation of a radical initiator such as 2,2'azobis (isobutyronitrile) (AIBN).

Many molecularly imprinted polymer studies conducted so far have aimed to explain organic polymers prepared by radical polymerization.

Monomers used in the preparation of organic polymers:

-Basic (vinylpyridine)
-Acidic groups (methacrylic acid)
-Charged groups (3-acrylamidopropyltrimethylammonium chloride)
-Hydrophobic (styrene)
-Hydrogen bonding (acrylamide) etc. can be counted

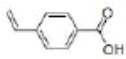
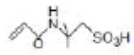
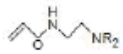
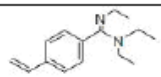
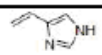
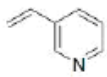
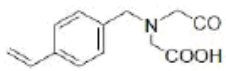
3.2. Advantages and Disadvantages of Different MIP Preparation Methods

Covalent and non-covalent imprinted methods both have advantages and disadvantages (Haupt, Cormack, & Mosbach, 2002). Today, many researchers use the non-covalent approach to prepare imprinted polymers (Kriz, Ramström, & Mosbach, 1997),(Zhong, Byun, & Bittman, 2001). The non-covalent approach is trouble-free and straightforward, as it allows the arrangement of functional monomers around the template molecule with non-covalent interactions prior to polymerization. Covalent modification of the template molecule is not required, and different binding interactions can be used to form the template monomer complex. Non-covalent binding kinetics are similar to enzyme-substrate binding compared to covalent binding (Boerje Sellergren, Lepistoe, & Mosbach, 1988). Imprinted polymers can be prepared without requiring specific knowledge of the structure and reactivity of the template molecule. However, interactions between the template molecule and functional monomers in non-covalent imprinting are not specific. The distribution of binding sites in non-covalent polymers is heterogeneous, resulting in nonspecific binding and low molecular recognition of the template molecule (Katz & Davis, 1999), (Dong et al., 2002). The stable template-monomer complex is formed before the pressing process in the covalent approach, so homogeneous distribution of the binding regions is provided (Figure 19) (Ikegami, Mukawa, Nariai, & Takeuchi, 2004).

3.3. Functional Monomers Used in Molecular Printed Polymer Preparation

The active center is formed from the reaction of a free radical with a monomer molecule. A linear polymer chain arises with the rapid addition of the monomer to the active center. The selection of functional monomers is critical, as the back bonding step between the template molecule and functional monomers is critical in molecular imprinted polymers. The key element for the functional monomer is the number of binding sites available for interaction to occur. The most commonly used functional monomers in the synthesis of MIPs are given in Table 3.

Table 2. Functional monomers and their structures

Functional monomer	structural formula of the monomer
Acrylic acids (R=H, CH_3, CF_3, CH_2COOH)	
Vinylbenzoic acids	
Acrylamidosulfonic acids	
Aminometacryl amides	
N, N Diethyl-4-Vinylbenzamine	
Vinylimidazoles	
Vinylpyridines	
4- (Vinylbenzyl) iminodiacetic acid	

In non-covalent molecular suppression, carboxylic acid groups, which are also found in the methacrylic acid structure, are used due to their ability to form hydrogen bonds (Lanza & Sellergren, 1999).

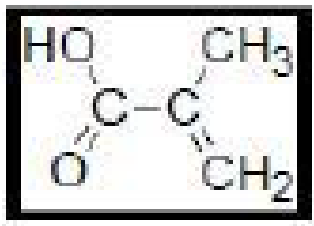


Figure 14. Methacrylic acid (MAA) structure used in non-covalent molecularly imprinted polymer preparation

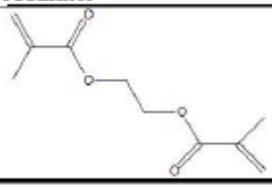
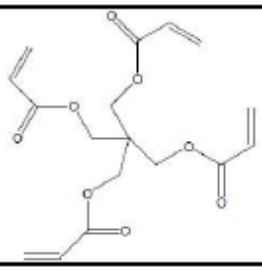
Methacrylic acid can act as an acceptor and donor while forming hydrogen bonds, and this property is advantageous for the retention of many analytes in back bonding studies (Börje Sellergren, 2000). Although MAA can make strong interactions with basic functional groups, its ability to form hydrogen bonds is not very strong in polar solvents. For this reason, acrylamide monomers are preferred as functional monomers instead of MAA in molecular repressions performed in solvents more polar than chloroform such as acetonitrile. Although acrylamide is less acidic than MAA, it is more polar and forms strong hydrogen bonding in polar media with the template.

3.4. Cross-linkers Used in Molecular Printed Polymer Preparation

The selection of cross-linker is another important point when preparing MIP. The cross-linker is used to form a durable polymer mesh that retains the analyte after the mold has been removed from the polymer (Idziak, Benrebouh, & Deschamps, 2001).

The list of commonly used cross-linkers is given in Table 3. Ethylene glycol dimethacrylate (EGDMA) is the most used cross-linker.

Table 3. Cross-linkers and Their Structures

Crosslinker	Molecular structure of the crosslinker
Ethylene glycol dimethacrylate (EDMA)	
p-Divinylbenzene	
Pentaerythrol tetraacrylate	

In MIPs, analyte selective cavity formation in the polymer network depends on the cross-linker / functional monomer concentration ratio and the appropriate cross-linker. Different cross-linker / functional monomer concentration ratios result in different numbers of binding sites in molecularly imprinted polymers, affecting selectivity (Dickert & Hayden, 1999).

3.5. Solvents Used in Molecular Printed Polymer Preparation

It is generally prepared and used in solvent media based on MIPs. For this reason, solvent selection is an essential point in the synthesis of MIPs. The solvent enables the formation of pores and temperature distribution and dissolving the polymerization components, having all components in a single phase. Generally, MIPs using polar organic solvents for synthesis, and MIPs using non-polar organic solvents

have worse selectivity (Yu & Mosbach, 1997). Whether the gap formed for the prepared polymer's selectivity to the analyte is the exact size of the analyte, neither too narrow nor too loose, depends on the choice of solvent. Besides, MIPs show different swelling properties in different solvents. Since swelling changes the functional groups' three-dimensional structure, which causes the MIP's selectivity to change, weaker bonding occurs in the analyte recognition step.

4. CONCLUSION

Polymers, biosensors, and molecularly imprinted polymer-based biosensors are investigated extensively in this graduation project. As mentioned before, biosensors are electronic devices that detect bioactivity in different conditions and environments. They have a wide usage area such as sample extraction, biological element recognition, metal ion detection, etc. In the COVID-19 pandemic, the SARS-CoV-2 virus is detected using biosensors, and due to the new studies, the portability of biosensors is provided. This makes the virus detection easy and intervention very fast. The importance of biosensors is covered in this project. In biosensor technology, there are different techniques. Each technique has unique advantages and disadvantages. One of the disadvantages is the reusability and reproducibility problem. Most of the biosensors are using organic, biological templates. These templates have high selectivity. However, their reusability and reproducibility are difficult because animals are used for obtaining these biological molecules, which brings ethics to

the agenda. By using molecularly imprinted polymers, reusability and reproducibility can be solved. MIPs are new and exciting technology in which polymers are used for the template. The template is formed using a target analyte for only one time, and the degradation of the polymer template is very low compared to the biological compounds. Especially for specific analytes, MIP technology becomes a low-cost technique.

MIPs have high reusability and reproducibility. Thanks to the new research, they also have high affinity and high selectivity. These unique advantages make MIPs very attractive for biosensors. In the introduction part of this graduation project, first, polymers are explained. Polymers have a wide usage area from daily-life applications to academic studies. Their chemical and physical bond interactions can be useful for biosensor technologies. Different polymers can be used, and polymerization techniques can also change the final properties of the polymers. After that, biosensor systems are explained. Biosensors' working principles, application areas, and also polymer usage in different biosensor systems are investigated. One can be said that polymer materials have high contributions to biosensor technologies. Finally, molecularly imprinted polymers are explained. MIP usage with different transducer types, advantages and disadvantages, usage areas, preparation techniques, and characterization of MIP materials is presented extensively. Studies clearly show that MIP materials can be used as biorecognition elements very well. Polymers' high binding properties and compatibility with other parts of the biosensor systems make them

excellent candidates for future biosensor technologies. There is an overview of recent and trend MIP biosensor systems in the below table with respect to used material, preparation technique, transducer type, and analyte to be detected.

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BÖLÜM 3

BIOTECHNOLOGICAL REVOCERY OF RARE EARTH ELEMENTS (REEs)

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1. INTRODUCTION TO RARE EARTH ELEMENTS (REEs)

Rare earth elements (REE) are a set of seventeen metallic elements that include yttrium and scandium, as well as the 15 lanthanide elements. Lanthanides are a set of elements with atomic numbers ranging from 57 to 71 that are chemically linked. Yttrium (atomic number 39) and scandium (atomic number 21) are also used in this group due to their chemical similarity to the lanthanides. Due to their comparable ionic widths and short atomic diameters, these two elements are found associated with rare earth elements in rare earth element mineralization (Zhang, Zhao, & Schreiner, 2016). REEs are transition metals from Periods 4, 5, and 6 of the periodic table's d-block. The lanthanide elements are split into two groups based on their solubilities in two salts: light rare earth elements (LREEs) and heavy rare earth elements (HREEs). The LREEs (atomic number Z=57 to 63) cover cerium, lanthanum, praseodymium, neodymium, samarium, and scandium, while the HREEs (atomic number Z=64 to 71) cover europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, lutetium, and y When considering their comparable physical and chemical characteristics, as well as their co-occurrence in nature, yttrium is often classified alongside the HREEs (Voncken, 2016).

Table 1. REE's atomic numbers and abundance on earth crust (Asdrubali, D'Alessandro, & Schiavoni, 2015).

ELEMENTS	ATOMIC NUMBER	ABUNDANCE (PPM)
YTTERBIUM	70	2.0
LUTETIUM	71	0.4
TERBIUM	65	0.7
EUROPIUM	63	1.3
GADALINIUM	64	4.0
DYSPROSIUM	66	3.8
PRESEODYMIUM	59	6.7
NEODYMIUM	60	27
HOLMIUM	67	0.8
ERBIUM	68	2.1
THULIUM	69	0.3
PROMETHIUM	61	10^{-18}
SAMARIUM	62	5.3

2. APPLICATION OF RARE EARTH ELEMENTS

REEs offer an extensive range of uses as their unique physical and chemical properties (Figure 1). REE applications and uses could be examined into two categories: traditional and high-tech. Metals and machinery, glass and ceramics, catalysts, magnets, and phosphors are among the traditional industries. The consumption of REE in these places accounts for roughly 85% of overall REE consumption (Balaram, 2019).

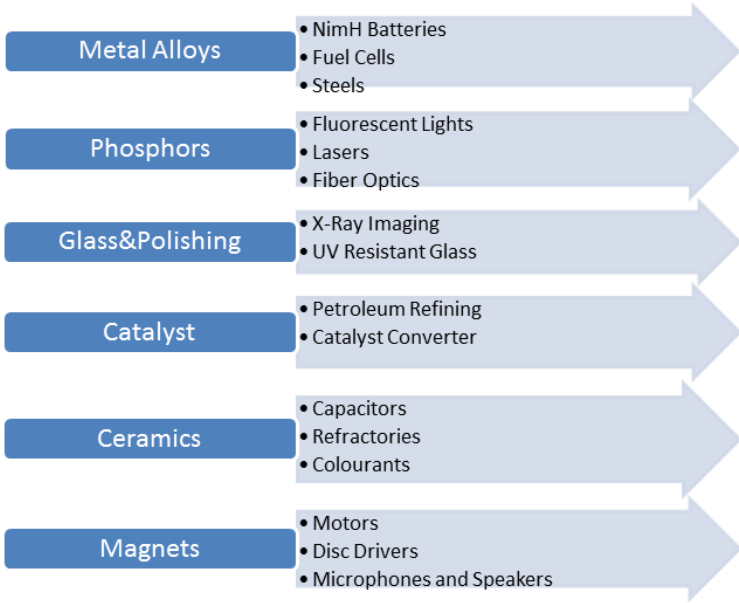

Figure 1. Rare Earth Elements Usage in Various Technologies (Balaram, 2019).

REEs are employed as mixed rare earth metals and alloys, as well as rare earth oxides and salts, in conventional applications. Phosphors, permanent magnets, batteries, and nuclear power, as well as superconductive and magnetostrictive materials, have all lately gained in popularity (Balaram, 2019). REEs aren't the primary component of the items in which they're employed. They are used as a product additive. Despite their modest volume and weight in materials, REEs are essential for devices to work. For example, varying amounts of Nd, Tb, Dy, and Pr are used in the construction of magnets, and while they only account for a small part of the overall weight, desktop and laptop spindle motors, voice coils, and other devices would be impossible to imagine without them (Tsamis & Coyne, 2015).

3. PERSPECTIVES OF REE RECOVERY AND RECYCLING

Fluorescent powder from end of life lamps is a viable solid matrix as a starting material for REE recycling. It includes yttrium, europium, and terbium, three of the five most critical rare earth elements, in gram amounts per kilogram of powder (Beolchini, Fonti, Dell'Anno, Rocchetti, & Vegliò, 2012). Advanced recycling operations would be relevant because the separation and recovery of lamps is currently necessary in many countries such as China United States and Australia, primarily to carrying away dangerous mercury from the wastes. Chemical techniques have largely covered the state of the art technology for REE leaching from lamp phosphors as of now (Peelman, Sun, Sietsma, & Yang, 2016)leachle. Firstly the phosphor mixture is chemically treated to dissolve the REEs, which can then be separated by precipitation or solvent extraction to extract REEs from lamp phosphors. REE mobilization following extraction from wasted fluorescent lamps appears to have a lot of potential for closing the loop product life industry and assisting to a more retainable future. On the top of that, by executing extraction and recovery processes by the aid of microbes (ferrooxidans, thiooxidans) and other type of bacterias (Figure 2) (komatogateibacter xylinus, lactobacillus casei, (Brandl, Barmettler, Castelberg, & Fabbri, 2016) with yeats (Yarrowia lipolytica) and tea fungus kombucha due to their buffering and electrochemical properties are relatively low-cost methods for waste materials treatment as well as it can also be used to a variety of leachable waste materials. That is why recent project about REEs

mobilization from fluorescent phosphors is applied by those (Hopfe, Kutschke, Pollmann, & Möckel, 2015).

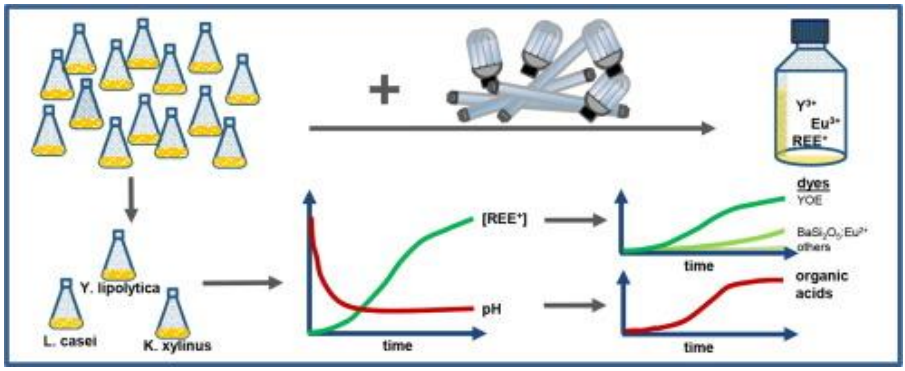

Figure 2. For recovery of REEs from fluorescent lamps by bacteria (Hopfe et al., 2018).

Therefore, bioleaching is assumed to be a much cleaner, efficient and inexpensive method for mobilizing rare earth metals unlike chemical leaching, that has a superior energy necessity to form high temperatures and generates chemical wastes. In constrast to common chemical treatment methods for recovering, bioleaching is a green environmental technology for REEs from industrial wastes plus its low cost will be expanding the area of usage in near future.

4. BIOLOGICAL PERPECTİVE FOR RECOVERY OF SELECTIVE RARE EARTH ELEMENTS FROM SOLUTION

Microbial cells have a significant propensity to REEs, as is popularly publicized. Microorganism systems are capable of acquiring metals as heavy bio-mineral deposits and other via simple biosorption (Lynne E Macaskie et al., 2009). Enzymatically, metallic ions such as UO_2^{2+} and La^{3+} could be transformed to metal phosphate minerals like HUO_2PO_4

and $LaPO_4$. Particularly under carbon restriction, it could up-regulated an acid phosphatase by executing a paradigm system. In response to diverse cell stressors, the phoN gene, which is controlled by the phoP/phoQ regulon, is up-regulated. The phosphatase is delivered and through the cell external surface that are periplasmic space and outer membrane, eventually settling in the extracellular polymeric matrix (EPM), which is made up of extensively hydrated polysaccharide chains from cell membrane material and certain lipids produced (Byeong C Jeong, Hawes, Bonthrone, & Macaskie, 1997). The phosphatase (Figure 3A) is bonded and assisted by the EPM which releases inorganic phosphate to settle with metal ions when provided with a sufficient organic phosphate molecule. As well as, nucleation sites are generated. Accordingly, the EPM builds the growing metal phosphate bio-mineral (Figure 3B), which develops as more metal and phosphatase substrate is added. The cells and the biofilm (Figure 3D) are coated by heavy mineral deposits in the end in the case of a biofilm (Figure 3C). It has to be known that the bacteria can be non-alive because the enzyme is basically immobilised; once the enzyme is generated and transferred into the EPM, metabolism is no longer required. Heavy mineral deposits arise because phosphatase activity is constant (Figure 3D). Bacteria produce biofilms on surfaces such as reticulated polyurethane foam (Figure 3E and F), which connect the bio-mineral (Figure 3G) to the flow within flow-through columns (Figure 2) (L E Macaskie, Lloyd, Thomas, & Tolley, 1996)(L E Macaskie, Jeong, & Tolley, 1994)(Tolley, Strachan, & Macaskie, 1995)lant. Although applying La^{3+} and cells encapsulated in a gel is

so effective as continuous metal extraction was described (L E Macaskie et al., 1996), REE bio-recovery industry has focused on the extraction of Nd^{3+} and Eu^{3+} previously because La is not a particularly significant REE. Initial experiments proceeded a gel-immobilisation technique, in which bacteria were cultivated as free cells in batch culture and then immobilized as well as A continuous filter was created by placing shredded gel into a glass column. Subsequently, biofilm-immobilized cell tests (Figures 3G and 2) employed columns made up of eight biofilm-coated polyurethane reticulated foam discs layered on a glass column with solution pumped upwards. Rare earth ions (REE^{3+}), glycerol 2-phosphate which is 5 mM for phosphate donor to provide inorganic phosphate for the developing mineral, and citrate buffer which is 2mM were added to the solution to achieve the desired pH. The quantity of metal chelation provided by citrate. This plays a critical role when comparing the extraction of various metals in the same solution matrix (Murray, Singh, Vavlekas, Tolley, & Macaskie, 2015).

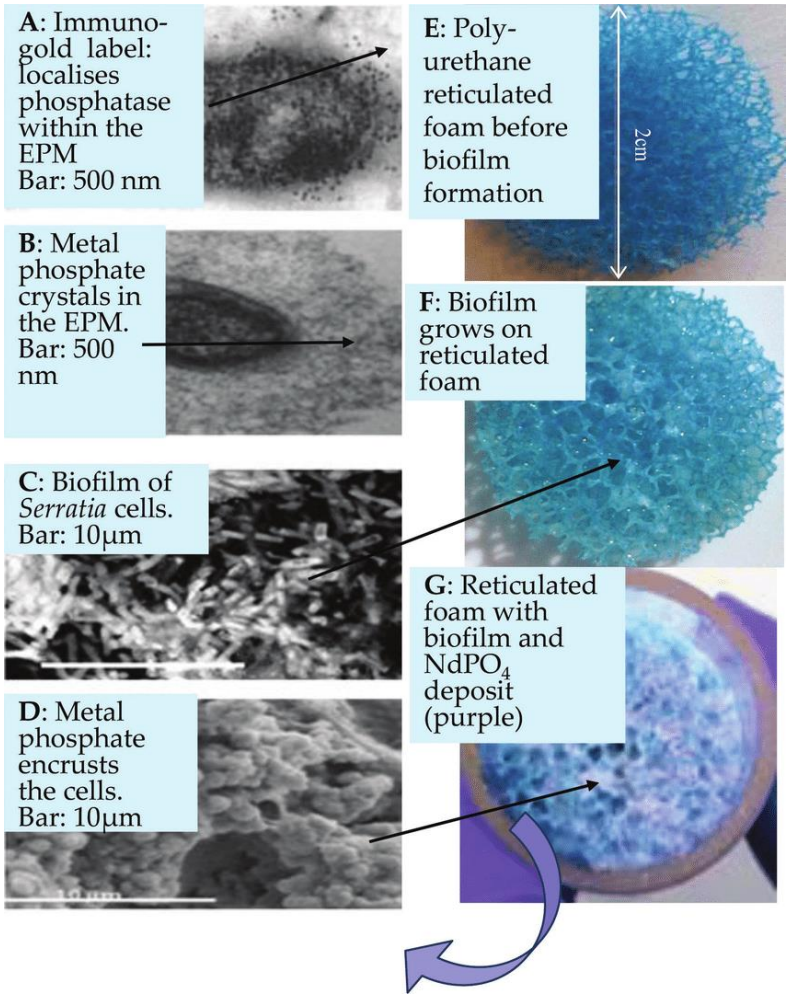

Figure 3. Serratia sp. cells establish phosphatase inside the extracellular polymeric material (A) and deposit metal phosphate in EPM (B). (C and F): biofilm-coated reticulated foam discs that have been created. (E): prior to biofilm settling, foam disc (D and G) NdPO4 deposition after passing a 1 mM Nd3+ and 5 mM glycerol 2-phosphate solution through a column (seen end-on in G) (Lynne E Macaskie, Moriyama, Mikheenko, Singh, & Murray, 2017).

The flow rate activity association could be used to define a working column. FA1/2 is the flow rate that allows 50 percent of the metal to be removed from the solution to remain in the solution. The full version of the Michaelis-Menten equation is used to quantify column activity, and it is exactly characterized as a plug flow reactor. Furthermore, the 'inefficiency' factor for the immobilised enzyme component is a lumped parameter that represents the combined effects of the metal's dissociation from its citrate compound and its extraction from solution into the precipitate. The Michaelis-Menten equation also explains the 'inefficiency' factor of the immobilised enzyme component, which is a lumped parameter representing the combined effects of the metal's dissociation from its citrate molecule and its extraction from solution into the precipitate. The extraction situation can be considered as the metal's tendency for forming phosphate deposits (Lynne E Macaskie, Empson, Lin, & Tolley, 1995). The actual times taken to achieve the multiple equilibria, on the other hand, do not need to be specified because they are dynamic in the flow-through system. Non-flow-through systems give essential data (Pettit & Powell, 2006). The net result as a quantity of metal extracted under a given situation is the result seen for column activity but it is possible that competing reactions could arise in some instances. By applying ^{31}P nuclear magnetic resonance (NMR) spectroscopy for instance, the phosphatase demonstrated activity of transphosphorylation which is the ability to produce substrate again. By trial and error method, it has been discovered that columns need a substrate for metal ratio of greater than 2:1. In the extraction of

uranium from solution, the predictive model's suitability has been discovered (Lynne E Macaskie et al., 1995)(B C Jeong, Kim, & Macaskie, 1997). Same model has been applied to extract uranium mine that also contains surplus sulphate ions in acidic wastewater subsequently (Lynne E Macaskie et al., 1997). To keep extraction efficiency at a high degree, slowing the flow rate by an amount that can be expected in a specific system are capable of accomplishing abundance of sulphate, nitrare and another flow components initiative degree (Yong & Macaskie, 1999)(Yong & Macaskie, 1997). In similar method, the solution's impact pH can be determined (Vavlekas, 2017). If the principal solution components and pH values are specified, the volume of the column which is necessary for a certain operation can be predicted using this modeling. Another important detail, the phosphatase enzyme does not have the activity as before during storage which is nearly 3 months when the activity of various cell preparations varies only by a few percent (Byeong Chul Jeong, 1992). A column series with new columns introduced as needed would be ideal technically. Typical inhibitors have no effect on the phosphatase enzyme, has a high sensitivity to fluoride, that is found in REE minerals as well as the newly explored mineral parasite yet (Jambor, Roberts, Owens, & Grice, 1996).

The extraction of REE3+ from solution was studied between polyacrylamide gel-immobilised free cells which were grown before in carbon-sufficient fed- batch culture and biofilm (from carbon-restricted culture). Free cells cultivated batch-wise have a 10-fold lower phosphatase activity than biofilm cells, they are about 250

and 2500 nmol product/mg protein per minute. The $FA_{1/2}$values for REE_{3+} extraction were 3.9 and 45.6 mL/mg cells per hour, respectively, indicating a 15% performance decrease by the gel-immobilized cells, implying a diffusional limitation within the gel (Lynne E Macaskie et al., 2017).

4.1. BIO-MINERALISED COLUMNS FOR RECOVERY OF REE

Extra processing operations would be required for REE bio-refining into new materials. Because NH_{4+} has been demonstrated to leach REE from clays and metallic wastes, it has a potential of using to extract REE from the bio-REE phosphate bio-material (Jun et al., 2010)(Moldoveanu & Papangelakis, 2013). Bio-extremely $REEPO_4$'s nano - crystalline structure can get it more 'friendly' to leaching than the strong acids necessary for 'geological' monazite minerals (Moldoveanu & Papangelakis, 2013). A sequence of tests utilizing columns supplied by acidic 100 mM $(NH_4)_2SO_4$ (to approximate leach liquid) did not reveal any impact on column activity at pH 5.5, with only a minor decrease in activity which is nearly 10% after exposure to pH 3.5 and recovery to pH 5.5. After each use, columns were returned to their original circumstances to ensure that their potential for utilizing ageing in many cycles (Murray et al., 2015).

5. REMOVAL OF REEs FROM WASTEWATER BY ALGEA

Algae have attracted a lot of interest in the Rare Earth Metals removal because of their efficiency, affordability, and environmental friendliness. As well as several research have looked into algae's ability to remove REEs from wastewater due to their unique characteristic properties. Adsorption onto the cell membrane and absorption into the cell are the two ways by which REEs are eliminated by algae, according to most studies. Adsorption is a metabolism-independent phenomenon in which algae and REEs generate new covalent and ionic bonds using various interactions, allowing REEs to be delivered between solutions and the surface of algae (Cao et al., 2021).

5.1. ADSORPTION MECHANISMS OF REEs BY DRIED/LIVING ALGEA

In wastewater that contains REEs, dried or living algae are discovered to consist of REEs on the surfaces, and REEs are transferred between solution algae surfaces via adsorption process. Adsorption is frequently a multi-step process involving multiple elementary processes that occur simultaneously or sequentially. The key adsorption fundamental pathways between algae and REEs cover;

i. surface complexation
ii. Ion exchange
iii. Electrostatic attraction
iv. Physical adsorption (Figure 3) (Cao et al., 2021)

As the kinds of interactions occurring, these basic mechanisms are classified into chemisorption and physisorption. Chemical adsorption involves surface complexation and ion exchange, in which new chemical bonds are formed by chemical reactions; physical adsorption involves electrostatic interactions or van der Waals, which cover physical adsorption and electrostatic attraction (Kegl et al., 2020).

Initially, it was difficult to differentiate between chemical and physical adsorptions, and numerous ideas, such as the D-R model and the amount of enthalpy change (H), were proposed. The estimated ordinary adsorption energy for the D-R model is less than 8 kJ/mol, suggesting physical adsorption, and between 8 and 16 kJ/mol, indicating chemical adsorption. Physical adsorption is shown in the range of 2.1 20.9 kJ/mol for the ΔH model, whereas chemical adsorption is shown in the range of 20.9 418.4 kJ/mol (Şeker et al., 2008)(Deng, Su, Su, Wang, & Zhu, 2007).

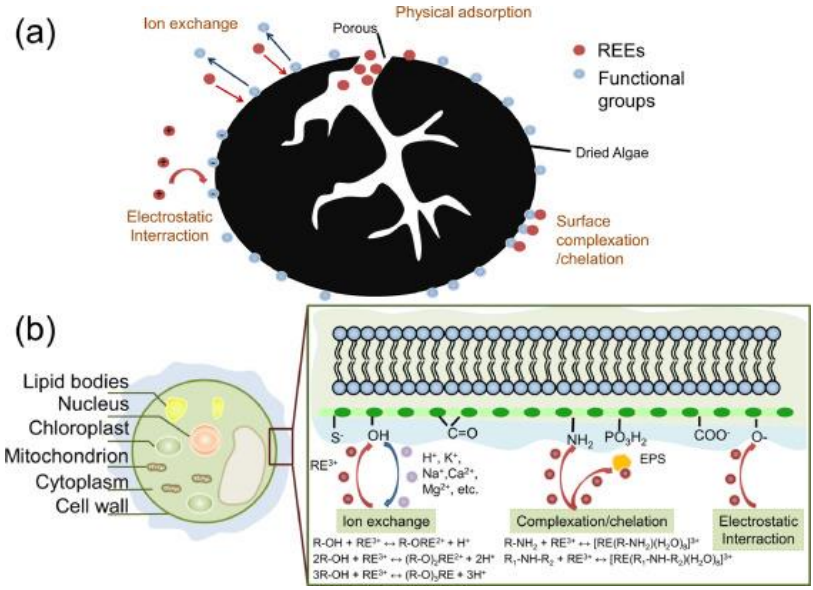

Figure 4. Representation diagram of the potential adsorption mechanisms of REEs by algae (alive and dried) (Cao et al., 2021).

Intermolecular movement, especially dispersion forces or Van der Waals forces, could be attributed to physical adsorption. Dried algae is quickly reproduced while maintaining the original structure due to the poor association between dried algae and REEs. It could be stated that, physical adsorption process which tends to achieve rapid equilibrium, is responsible for the preliminary quick removal of REEs by alive/dried algae. Physical adsorption is aided by the big surface precise area and cultivated porous assembly, particularly micro pore arrangement (Das & Das, 2013).

There is an electrostatic attraction that dweels between the negatively charged functional groups on algae's surface and positively charged REEs. The surface of algae cells has been found as robust electron-

donators and weak electron-acceptors. The external charge of algae cells, on the other hand, is mostly determined by the solution pH, taht affects whether REEs and algae interact via electrostatic repulsion or attraction. Due to electrostatic repulsion the positively charged surface of the algae limits REE absorbing, when the solution pH is less than the pH point of zero charge (pzc) of the algae. As well as, the negatively charged surface of algae holds REEs by electrostatic attraction when the solution pH is greater than the pH pzc of the algae. In addition, electrostatic attraction is also determined by expansions in the nuclear charge of REEs, not just by ionization of algea's surface groups (Hein, Pedersen, & Sand-Jensen, 1995).

Ion exchange appears between light metals like Na^+, K^+, H^+ and REEs on the cell membrane of algea and van der Waals forces, electrostatic forces, and the generation of ionic or chemical covalent bonds can be emanated from key interactions. Since REEs in solution substitute the same volume of other ions on the algae's surface, algae confirm and measure the ion exchange adsorption of RE metals (Ramasamy, Porada, & Sillanpää, 2019). However, the formation of major levels of H+ virtually protonates functional groups, resulting in a reduction in binding sites on the algae surface, putting REEs at a disadvantage in reasonable adsorption at low pH (Vijayaraghavan, Sathishkumar, & Balasubramanian, 2011).

Complexation emerges when REEs rejoin with functional groups of algae to generate complex structures, such as the reaction of REEs with amino groups. When investigate the complexation process due to

atomic weight of REEs, some heavy and middle REEs tend to complex with carboxylate while middle and light REEs are more inclined to be complexed with phosphoryl groups. The construction coefficients of REEs complexes are connected to the narrowing of ionic radius of REEs, according to earlier searches, and the formation constants in the REEs series climb from La to Lu. The mechanism's fundamental interactions include covalent, electrostatic, and coordination interactions. The adsorbed REEs are stabilized by the complexation mechanism enhanced than by further mechanisms, creating them less possible to dissociate from the algae's interface (Crane & Sapsford, 2018)(Bulgariu & Gavrilescu, 2015).

Ionic strength which is subject to behavior generates a wealth of information about the complexation mechanism, that could be categorized into outer-sphere (electrostatic) and inner-sphere (ligand exchange) complexation mechanisms as regards the negative or positive correlation between adsorption capacity and ionic strength. In previous studies, it has been detected that, six biotypes of living macroalgae all removed more REEs in a solution by a salinity of 10 than 30, suggesting that the adsorption method was mediated by the outer-sphere complexation process (McBride, 1997).

As well as, the associations among functional groups of algae and REEs are related to the soft and hard bases and acids (HSAB) 39principle, in which acceptor-donor connections are either soft-soft or hard-hard, resulting in strong interactions. Because an oxygen atom is a hard base according to the HSAB principle, complexation

between oxygen-enclosing functional groups on the algal surface and hard ions like REEs occurs smoothly (Pearson, 1990)(Ramasamy et al., 2019).

On the top of that, the adsorption procedure on alive/dried algae is controlled by basic mechanisms (Figure 3), with the most significant aspects determining the category of straightforward mechanisms;

1. the environmental situation of wastewater

2. the series of REEs in wastewater

3. the types of functional groups on the interface of algae

5.2. PREOCCUPATION (INTERCELLULAR ACCUMULATION) MECHANISMS OF REEs BY ALIVE ALGEA

REEs linked to cell walls might be carried inside cells by transporter proteins throughout the metabolism of algae cells, a process called as preoccupation (intracellular accumulation) for alive algae (figure 5). REEs that are taken inside algae cells could bind to peptide ligands, proteins, and further molecules. Metal-binding ligands like metallothioneins and phytochelatin have been discovered in the cells of Chlorella vulgaris (Lederer, Curtis, Bachmann, Dunbar, & MacGillivray, 2017).

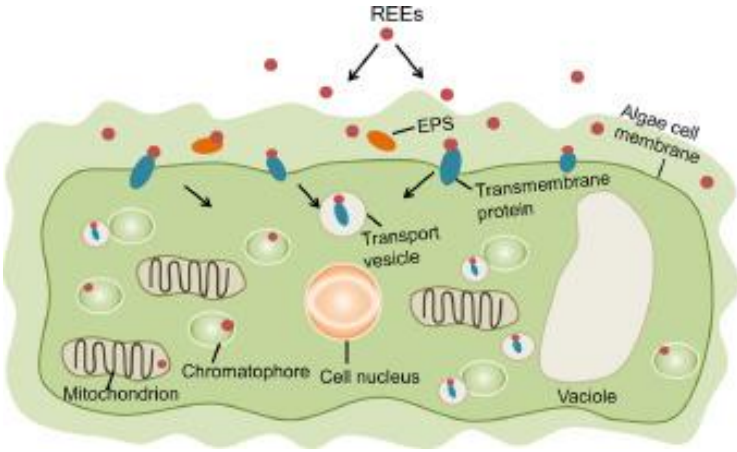

Figure 5. REE absorption diagram for living algea [29].

Heavy metals taken into Chlorella vulgaris cells are capable of attaching to phytochelatins in the cytosol and subsequently remain delivered to acidic vacuoles, allowing of the heavy metal for unleash or separate as well as return of peptides to the cytoplasm. This mechanism can also be applied for the preoccupation of REEs with living algae because REEs get assembled in cellular sections (Řezanka et al., 2016).

Moreover, it could be stated that, the absorbed REEs have a significant function in living algea's growth co-factors and metabolism. Location of Ce and Nd in the chloroplast and location of Gd and La in the cytoplasm have been found in previous study (Kang, Shen, & Jin, 2000). There are various researches which indicate that REEs can gather in different plants chloroplasts and it might be true for the same mechanism on algae cells. La and Gd can aid in the

maintenance of the cytoskeleton and play a role in cytoplasmic signaling ways (Liu & Hasenstein, 2005).

Lower concentrations of REEs have also been shown to boost the action of peroxidase, superoxide dismutase, and catalase in algae that have been seriously affected by heavy metals. They reduce oxygen to hydrogen peroxide and oxidize O2 to its algae cells to remove Reactive Oxygen Species (ROS) and reduce oxidative damage. REEs are moved to various structures within algae cells and have varying impacts on their composition, that are mostly dependent on the proteins and metabolic components found within algae cells (Yang, Yingjun, Jinge, Zhanghong, & Qinglian, 2016).

It had been put forward that algae decreased toxic indications by combining REEs with a large number of biological macro-molecules, causing REEs to generate stable complexes with biological macromolecules by compensating bivalent cations like Ca^{2+} in a variety of functional bioreactions. It is absolutely verified that REEs link with phosphoproteins and Ca-binding proteins in Galdieria sulphuraria which is a red algea by living algea. Another theory suggests that Galdieria sulphuraria algea generate porphyrins which are biological cells for absorption of La(III) because algae's biological complexes contain greater assimilation for La(III) and Cu(II) as well (Minoda et al., 2015).

On the other hand, algae's capacity to absorb REEs inside a particular concentration range could be by virtue of their cell wall barriers and homeostatic systems are not activated or adequate to prevent REE

adsorption. The complex polysaccharides in algae cell walls, have been found to constitute a beneficial barrier to prevent REEs' harmful impacts, resulting in reduced REE absorption conversely. The absorption of REEs by alive algae might be restricted because of reduced solubility of REEs in lipids. In the light of all these information, we can clearly state that more investigations and projects need to be done in detail for future (Ochsner et al., 2019).

6. REMOVAL THROUGH MICROBIAL CELL SURFACE ADSORPTION (BIOLEACHING)

One of the most promosing green technology and low cost suitable method for extracting Rare Earth Metals is bioleaching. Microbial action consequences in metabolites that dissolve and release metals from a solid matrix, that can be either ores or wastes. These type of biohydrometallurgical processes have lower costs for mining companies and improved ecology friendly to the world. Microorganisms which can oxidize sulfidic raw materials in the ore to form acid for metal dissolution make this process happen. Almost all REE wastes do not have sulfur element, bioleaching processes exploit heterotrophic microorganisms which extend on organic compounds to generate organic acids unlike copper bioleaching microorganism that generate ferric iron and sulfuric acid as well as gold recovery where microorganism are operated for dissolving the sulfidic mineral maxrix around the gold instead of direct solution of the target metal. These organic acids are very enviable to extract metals such as REE from

non-sulfur-containing waste materials (Brisson, Zhuang, & Alvarez-Cohen, 2016).

Various heterotrophic bacteria have been found to leach REE from end-of-life materials such as retorted phosphors and spent fluid catalytic cracking (FCC) catalyst; from these, Gluconobacter oxydans, a proven bacterium that produces organic acids such as gluconic acid by oxidizing carbohydrates NRRL B5810, has been identified as a potentially powerful strain. G. oxydans' lixiviant (spent medium comprehending exudate), which contains mostly gluconic acid, can remove about half of the total quantity of REE material present in FCC catalyst when grown with refined glucose (ASAI & SHODA, 1958). According to Life-cycle analysis (LCA) and Techno-economic analysis (TEA) for bioleaching of REE extracted from spent FCC catalyst, a bioleaching institution based on G. oxydans lixiviant generation would be absolutely more cost-effective and environmentally friendly than a chemical leaching factory (Thompson et al., 2018).

6.1. MICROBE REE INTERACTIONS

There have been a number of studies on microbes and REE interactions, including REE extraction from solids via metabolic processes, REE immobilization from liquids by biomass sorption, and the role of REE in bacterial proliferation. The adsorption behavior of Europium (Eu) was investigated using Gram-negative (thin peptidoglycan layer) and Gram-positive (thick peptidoglycan layer) Halobacterium salinarum, Pseudomonas fluorescens, and Bacillus

subtilis cell envelopes. Dysprosium (Dy) biosorption was seen even when a new fungus isolate, Penidiella sp. strain T9, reduced the pH to 2.5 (Horiike & Yamashita, 2015). Thereafter heavy REEs such as thulium, ytterbium, and lutetium were absorbed to a greater extent compared to light REEs as Roseobacter sp. AzwK-3b cells were pre-protonated by nitric acid at this pH (Bonificio & Clarke, 2016).

Acidolysis, redoxolysis, and complexolysis are the most common methods for microbially intervened element mobilization. Acidolysis which is known as proton-induced solubilization is the process of protons exchanging and replacing components on mineral surfaces. Redoxolysis refers to the process of mobilization via reductive or oxidative processes, while complexolysis refers to the reaction of complexing agents with mineral surfaces which is also called ligand-induced solubilization. These general mechanisms are valid at whole solid matrices, also at materials which consisting of REE. On the other hand, we must state in detail why microorganisms mobilize REE and find the ecological advantage of these from interaction. Unfournately, there is not various available reports and since the attention has been mostly on microbe-metal-interactions of market metals, there is a significant gap in investigations on the mechanistic relationships between microbes and REE. There is an opportunity for microbes to mobilize REEs bu just luck or there might be a need for these elements. Lately, some projects point, since REEs serve as necessary cofactors for some of the microbe's main enzymes, some bacteria are completely reliant on their existence for growth. Methylacidiphilum fumariolicum grown on methane exhibited a favorable association

between growth and cerium concentrations. Although samarium, europium, and gadolinium supported growth to lower extent, lanthanum, neodymium, and praseodymium were more favorable. Therefore, further researches will be needed to see whether these findings are applicable and REEs can boost the growth of other bacteria too.

The introduction of lanthanum and cerium to Methylobacterium radiotolerans, M. fujisawaense, and M. zatmanii boosted the efficiency of methanol dehydrogenase by a factor of four to six when compared to calcium, implying the induction of dormant genes. REE-dependent proliferation was observed in mutant strains of Methylobacterium extorquens . Because of the rised activity of a lanthanide-dependent methanol dehydrogenase, lanthanum concentrations as little as 2.5 nM promoted growth on methanol compared to the presence of simply calcium. By considering all these studies, methylotrophic microorganisms could be useful and get an implementation area in the biomining and recycling of rare earth elements (Brandl et al., 2016).

6.2. MECHANISM OF REEs BIOSORPTION

Adsorption process has gotten a lot of interest to extract metals. Biosorption is a new biological approach that has a number of superiorities over traditional techniques. Bisorption technique generates no chemical sludge, is simple to use, and effective in removing pollutants from quite diluted solutions. On the top of that, the greatest benefit is that it could be used in situ and with appropriate

design. No industrial operations are required and it can be associated with several systems (Tewari, Vasudevan, & Guha, 2005). The main motivations for bio sorption mechanisms are their extreme activity for metal removal, inexpensive biosorbent prices, no production of secondary scraps, rapid kinetics and reuse of the biosorbents (Kratochvil & Volesky, 1998). As well as, metal binding moves along ion-exchange, surface complexation, electrostatic interaction, and precipitation this step may happen separately or connected in several mechanisms of bio sorption of REMs (figure 6) (Oliveira, Jouannin, Guibal, & Garcia Jr, 2011).

The mechanism of rare earth element bio sorption can be affected by properties of metal solution chemistry, kinds of biomaterials and environmental conditions. Basically bio sorption process by living cell contains two different steps. Metal ions are adsorbed to the cell surface in the first step due to relations among metals and functional groups on the cell surface. Whole metal ions get to meet the cell wall prior to have arrival to the cell membrane and cell cytoplasm. The cell wall is constituted by polysaccharides and proteins therefore it supplies a number of active locations which can attaches metal ions. There are considerable variances in the kind of metal ions that attach to them, as well as the amount of metal ions that attach to them because of cariations in the composition of cell walls between various microorganism groups such as algae, fungi, yeast, bacteria (Goyal, Jain, & Banerjee, 2003).

The utilization of brown algea Sargassum biomass for lanthanum connection has been investigated and presented previously. An amorphous matrix and a fibrillary skeleton are main parts of the cellular walls of brown algea. The external layer is an amorphous medium that is hydrogen-bonded to the fibrillar skeleton which contains mostly cellulose.

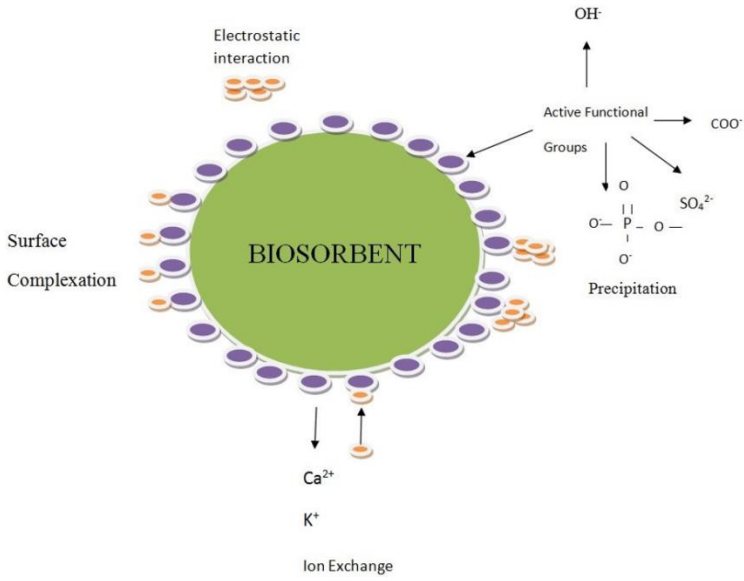

Figure 6. Representation of the several mechanisms of bio sorption of PEE.

Alginate makes up the majority of the amorphous matrix and small rate of fucoida which is a brown algea. The alginate makes the cellular wall more flexible and resistible. Metal bindings are mainly composed of alginate carboxyl groups as well as they form 70% of standardized parts. The quantity of carboxyl groups in the alginate polymer is related to biosorption absorption straight ahead. Sulfonic acid which exists in the fucoidan algea is available in the second functional group

of the brown seaweed. In addition biosorption process is promoted by these groups (Davis, Volesky, & Mucci, 2003).

There is majorly peptidoglycan made up linear chains of the disaccharide N-acetylglucosamine- β1, 4-N acetylmuramic acid on cell wall, plus it contains peptide chains that have been demonstrated for metal ions isolation. For lanthanum biosorption mechanism, several chemical characterization methods such as energy dispersive X-ray analysis (EDAX), Fourier-transform infrared spectroscopy (FT-IR), and X-ray diffraction analysis (XRD) have been used. FT-IR spectroscopy and XRD analysis identified that the effective participation of cellular carboxyl and phosphate groups occurs in La(III) absorption by microbial biomass. As well as, binding between lanthanum and bacterial biomass through shift of cellular calcium and potassium has been indicated by EDAX and elemental analysis. On the other hand, Transmission Electron Microscopy (TEM) has shown that The cytoplasm of the bacterial cell absorbs la, not the cell boundary (Hoyle & Beveridge, 1983)(Kazy, Das, & Sar, 2006).

Varied functional groups for adsorption of the metal ions was indicated by FTIR in bamboo charcoal for pre-adsorption and post-adsorption of La(III) (Qing, 2010). Thereafter chemical processing and metal adsorption upon that functional groups resulted in decreased and elimination of the peaks in FTIR spectra shifts.

When microalgae such as penicillium sp, monoraphidium sp, bakers yeast, and activated carbon effect on neomydium for biosorption mechanism was investigated two decades ago. Firstly, binding groups

in the microalgae cell wall contain carboxyl, amino, sulfate and phosphate anions and the variations in metal uptake were attributed to the organization, functional groups, and surface area of each microbe. Filamentous fungi's cell walls are primarily made up of amino or non-amino polysaccharides. The quantity of nitrogen within the polymer chains determines the capability of these altered polysaccharides to hold metals. But then, there is a protein shell which could provide a charge by decomposing ionizable side groups of the constituent amino acids on the exterior layer Saccharomyces cell wall (Xiong, Xinyi, & Caiping, 2011).

The resin samples of IR spectra have been processed before and after Yb(III) adsorption to explain the mechanism of Y(III) adsorption on gel-type weak acid 110 resins. Finally, chemical bonds were produced by the functional group of 110 resin (C=O) and Yb(III). Coordination interactions were created among oxygen atoms and Yb(III), according to the findings (Zheng & Xiong, 2011).

7. FACTORS EFFECTING THE RARE EARTH ELEMENTS' BIORECOVERY

The bio recovery of REEs is impacted by a variety of parameters, which could be divided into three categories: chemical factors, physical factors and microorganism kind. Chemical factors cover redox potential, pH, and metal toxicity while the physical factors cover aeration, temperature, and pulp density. All of these elements can act alone or in concert to influence the entire REE biorecovery mechanism.

7.1. PHYSICAL FACTORS

7.1.1. AERATION

Aeration plays a significant role for microorganisms development which takes part in REE biorecovery because Acidophilic chemolithotrophic bacteria that associate to REE biorecovery use O_2 as a terminal electron acceptor in their metabolism. As the bioleaching process, CO_2 and O_2, that serve as carbon foundation and electron acceptor for autotrophic microorganisms A. ferrooxidans and A. thiooxidans, respectively, must be provided appropriately by aeration. The selection of optimum aeration based on the process design and the individual microorganism such as A. ferrooxidans and A. thiooxidans and the sulfur oxidizing bacteria Sulfobacillus sp that are required to dissolve REE minerals in the process has a significant impact on the effective procedure of a bioleaching process (Dev et al., 2020).

7.1.2. PULP DENSITY

Pulp density is a key factor in microbial growth, that influences the metal biorecovery future. Because of the strong shear force, the high pulp density inhibits microbial growth by restricting CO_2 and O_2 flux and raising harmful metal contents. As well as, pulp density has an impact on pH due to its strong buffer ability (Arshadi & Mousavi, 2015).

7.1.3. TEMPERATURE

Temperature has a crucial part in the mechanism of REE biorecovery thanks to its significant impact on metabolic activity, microbial

growth and kinetics. Microorganisms are classed as mesophilic, psychrophilic or thermophilic created depends on their temperature desires, with optimum development temperatures about −4 -20°C, 25–47°C, or 41–68°C correspondingly (Zhao et al., 2018).

7.2. CHEMICAL FACTORS

7.2.1. pH

The pH of the aqueous solution influences REE solubilization and the development of microbes participating in REE bio recovery. The microorganisms engaged in REE biorecovery are commonly acidophilic. Table 2 shows their optimal growth pH with the temperature at which various microorganisms capable of bioleaching REEs grow efficiently. Microorganisms' generation of Extracellular polymeric substances (EPS) is similarly affected by pH. A large EPS composition is produced by an acidic pH, leading to greater microbe adhesion to the mineral surface and enhanced REE extraction (Wang et al., 2018).

Table 2. The optimal growing pH and temperature for the microorganism for bioleaching.

Microorganism	**Optimum temperature(°C)**	**Optimum pH**
Acidithiobacillus ferriredurans	29	2.1
Acidithiobacillus ferrivorans	27-32	2.5
Acidithrix ferrooxidans	25	3-3.2
Ferrimicrobium acidiphilum	35	2

Ferrithrix thermotolerans	43	1.8
Aciditerrimonas ferrireducens	50	3
Alicyclobacillus aeris	30	3.5
Alicyclobacillus ferrooxydans	28	3
Ferrovum myxofaciens	32	3
Thiomonas islandica	45	4
Sulfobacillus thermotolerans	40	2
Sulfobacillus benefaciens	38	1.5
Ferroplasma cupricumulans	53.6	1-1.2
Ferroplasma thermophilum	45	1
Acidianus manzaensis	74	0.8-1.4
Metallosphaera cuprina	65	3.5

7.2.2. REDOX POTENTIAL

The redox potential of the ambient aqueous system influences the dissolution of REE from a mineral stage by a microbe-facilitated redoxolysis technique. Iron-oxidizing microorganisms like as L.ferriphilum, A.ferrooxidans, A.thiooxidansor provide extreme major redox potential for the oxidation of Fe^{2+} and Fe^{3+}. In previous studies, A. caldus was used to extract 52 percent Sc, 52.6 percent Y, and 59.5 percent La from coal slag at a redox potential of 845- 855mV and throughout a bioleaching experiment employing discarded LED bulbs as a mineral source, A. ferrooxidans was shown to oxidize Fe2+ to Fe3+ at > 600mV. REE dissolution proceeds at a reduced redox potential due to the sulfur oxidizing bacteria-mediated acidolysis

mechanism (Pourhossein & Mousavi, 2019). It has been stated that at a redox potential of 100–150mV, Sulfobacillus effectively oxidizes sulfur (Yahya & Johnson, 2002). To achieve the necessary redox potential for enhanced REE biorecovery, it is critical to choose a microbial strain depending upon mineral properties.

7.2.3. METAL TOXICITY

The risky interior environment of waste materials might jeopardize the REE biorecovery process. Furthermore, heavy metals and minerals may stifle microorganism growth and metabolic activity by accumulating intracellularly, inhibiting particular enzymes, and deteriorating the membrane transport system. Heavy metal inhibitory effects can be avoided by altering and acclimating the inoculum to gradually growing metal concentrations and/or co-culturing with other microorganisms (Monballiu et al., 2015).

8. CONCLUSION

Some raw materials are considered as critical raw materials according to their supply risk and importance in the economy (EU commission 207 report), REEs are at the top of the critical raw materials. Political and strategic conflicts between countries bring great risks in the supply chain. However, the demand for REEs, which are used in many sectors, is constantly increasing depending on current and new technological developments. It is predicted that the world's annual REE demand will be 220,000 tons in 2025 and 350,000 tons in 2035[1]. If new resources cannot be found, there is a risk that there will be supply shortages in some REEs after this date and that the

supply will not be able to meet the demand.The need for rare earth elements, which are vital, is being driven by population growth, economic growth, and a desire for new technology. This expansion necessitates innovative rare earths production that is both secure and environmentally friendly. Hundreds of rare earth deposits are being developed around the world, ranging from discovery to confirmed mineral reserves and comprehensive engineering.

Due to their critical role in electronics, rare-earth elements (REEs) are becoming increasingly important in the transition to a green economy. The rest of the world confronts a REE supply risk since China now produces more than 90% of global REE output and has increasingly strict export restrictions. Mining companies are looking for new REE discoveries, and some old mines are being reopened. Many countries will have to rely on recycling and waste treatment of REEs from pre-consumer scrap, industrial wastes, and REE-containing End-of-Life items because to a lack of commercially or operational primary deposits on their territory. Furthermore, REE recycling and recovery from metallurgical wastes such as red mud, fly ash, electronic magnets can assist in reducing the environmental issues that come with REE mining and processing. Many countries will have to create strategies for waste treatment and recycling of Rare Earth Elements, given that new mining companies are exploring for new Rare Earth Elements deposits and existing enterprises are re-opening for this purpose. While efficient recycling pathways for basic metals (iron, copper, aluminum, zinc, etc.) and precious metals (gold, silver, platinum metals) have already been established but rare earth

recycling rates are still less than 5%. Thus an increase in research activity towards the establishment of better rare earth recycling methods must be introduced and, much higher than the current 5% number must be obtained in near future.

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BÖLÜM 4

NANOPARTICLES FOR BOMEDICAL APPLICATIONS

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1. INTRODUCTION

1.1. Nanomaterials

Nanomaterials are significant components of nanoscience and nanotechnology. Nanomaterial is an object with at least one dimension on the nanometer scale (~1-100 nm). Two types of nanomaterials can be found: (i) first one is naturally occurring nanomaterials which are proteins, viruses, nanoparticles from volcanic eruptions, etc., (ii) another one is engineered nanomaterials designed at the molecular level to gain advantages and novel properties due to their sizes. Examples of this type of materials are metal oxides, quantum dots, nanotubes, core-shell nanostructures, etc.

Nanoscience is the manipulation of materials and the study of phenomena at atomic, molecular, and macromolecular scales and an interdisciplinary field that includes chemistry, physics, biology, and materials science. In fact, nanoscience is the study of materials having at least one of their dimensions ranging from 1 to 100 nm (Dowling et al., 2004). The word "nano" refers to a scale of size in the metric system meaning "small". It is used in the scientific systems to denote one-billionth of a meter (1 m = $1x10^{-9}$ nm). For one example, a human hair diameter is approximately 100 micrometers which are 100,000 times bigger than 1 nm, and in another example, the diameter of a hydrogen atom is approximately one-tenth of one nanometer.

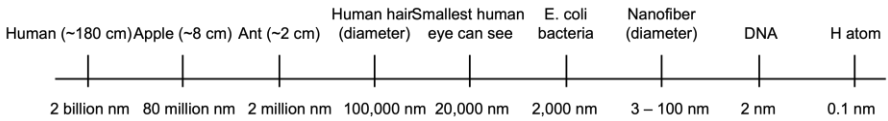


Figure 1. Comparison of sizes

Nanotechnology is concerned with the study and use of functional structures with at least one of the dimensions less than 100 nm and covers the construction of various materials to produce devices and products. The nanoscale structures allow them to have improved chemical, physical and biological properties due to their size. Considering quantum dots (QDs), QDs are low toxicity and biocompatible (based on the composition) nanostructures and exhibit different fluorescence properties because of changes in size. Studying their physical and chemical properties is nanoscience, whereas nanotechnology uses these materials in bioimaging, diagnostics, and biosensing applications (Cotta, 2020).

1.2. Classification of Nanomaterials

Nanomaterials are classified into two groups: (i) size-based and (ii) material-based. Classification of nanomaterials is illustrated in Figure 2.

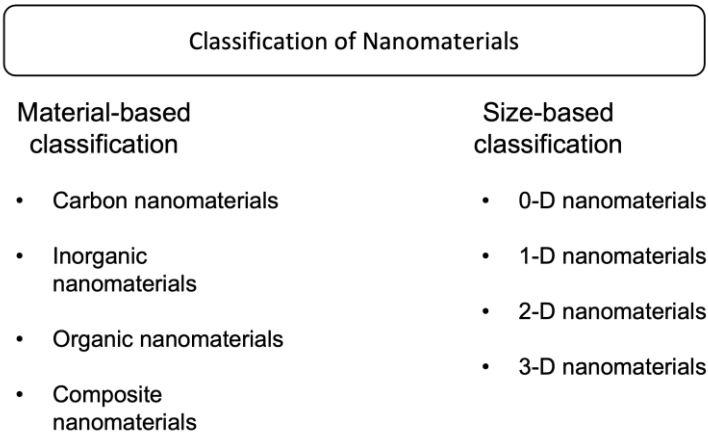


Figure 2. Classification of nanomaterials

1.2.1. Size-Based Nanomaterials

Zero-dimensional: All three dimensions of the materials exist at the nanoscale. Examples for zero (0) dimensional materials are gold, palladium, platinum, silver, quantum dots, nanospheres, fullerenes, core-shell or hollow nanospheres.

One-dimensional: One of the dimensions (length or width or height) of these nanomaterials is in the range of 1 to 100 nm, while the other two dimensions might be on the macro scale. This type of nanomaterials are nanowires, nanofibers, nanorods, and nanotubes. One-dimensional nanostructures can be made from metallic, ceramic, polymeric, and other materials.

Two Dimensional: Two dimensions are at the nanoscale and one dimension is at the macro scale. Nano thin-films, thin-film multilayers, nanolayers, or nanosheets are two-dimensional (2D) nanomaterials.

Three-dimensional: All dimensions are at the macro scale. Bulk materials and crystals are 3D nanomaterials.

1.2.2. Material-Based Nanomaterials

Carbon Nanomaterials: These types of nanomaterials consist of carbon content and have different morphologies such as hollow carbon nanospheres or nanofibers, fullerenes, and graphene.

Inorganic Nanomaterials: Metals (Ag, Au, Fe, etc.), metal oxides (TiO_2, MnO_2, SiO_2, etc.), and ceramics belong to inorganic-based nanomaterials.

Organic Nanomaterials: Organic-based nanoparticles are made of organic molecules such as polymeric materials, lipids, or carbohydrates.

Composite Nanomaterials: Composite-based nanomaterials are made of two or more layers of nanoparticles which can be form of carbon-organic, organic-organic, metal-carbon, metal-carbon-polymer, etc.

2. SYNTHESIS OF NANOSTRUCTURED MATERIALS

2.1. Top-Down

Top-down nanotechnology is a technology to produce nanostructured materials, in which nanoscale particles are obtained by grinding larger particles, powders or solid particles. In top-down processes, a bulk material is restructured to convert it into a nanomaterial form; for example, it is distributed, machined, processed, or precipitated. Ball

milling, and lithography are the most widely used top-down methods for nanomaterial synthesis.

2.1.1. Ball Milling

A ball mill is a system for grinding hard materials, mixing solids and liquids. The term ball mill covers a large group of devices with different design working principle. The grinding and mixing processes are done using balls of varying sizes and densities made with materials ranging from high-strength alloys and dense ceramics to plastics. The use of ball mills in industry is quite common; Grinding coal in thermal power plants and grinding in laboratories to obtain nanomaterials are two typical examples of using ball mills of various types. With ball mills, suspensions, and powders with particles smaller than 100 nm can be obtained. The distribution of particles is quite wide, so various separation techniques are applied to separate the grains.

High-energy ball milling is a method of producing nanopowders with an average particle size of fewer than 100 nanometers. To make a range of nanocrystalline powders, high energy ball milling is a simple, effective, and efficient method (Rane, Kanny, Abitha, & Thomas, 2018). Grinding generates micro deformation of the crystal lattice in the material during particle size reduction, and some of the energy is squandered on the development of micro stress, which slows down the grinding of the powder. In a liquid grinding media, the best grinding occurs (alcohol or other organic solvents). The high-energy ball milling method involves applying high-energy ball impacts to a

powder combination in a ball mill. The powder particles are subjected to a high energy impact during the procedure. The mechanical alloying process may be divided into four stages microstructurally: the start stage, middle stage, final stage, and completion stage: (a) The powder particles are flattened in the ball mill's initial stage by the compression forces caused by the balls colliding. (b) The 'cold welding' step, which plays a crucial role in particle size reduction, is the second or intermediate stage. The powder's chemical makeup is still not uniform. Particle size refinement and reduction is the final stage of the process, and the powder particles have a severely deformed metastable structure after it's finished.

2.1.2. Lithography

Lithography can be performed by applying light emission (photolithography), X-rays (X-ray lithography), electron/ion flux (electron-beam/ion-beam lithography) from a template to a special surface (polymer sheet, semiconductor substrate, etc.), or is an image transfer technique with scanning probe microscopy, atomic force microscopy or contact printing methods. The synthesized nanoparticles are produced with this method in the range of 1 to 100 nm. Historically, lithography is a method applied to transfer a previously prepared image on a flat stone surface or text onto a paper. Today, the term 'lithography' has a meaning of an image transfer technology. From the nanotechnology perspective, lithography includes several stages: (i) A photosensitive polymer film (photoresist) is applied on a silicon wafer, (ii) the film coating on the

wafer is dried and irradiated (with a suitable mask), (iii) the irradiated coating is etched in a solution, and (iv) a required shape is formed on the substrate. The main advantage of this technique is the synthesis of single-sized nanoparticles in a desired shape and size although lithography requires complex and expensive equipment (Kumar & Kumbhat, 2016b).

2.2. Bottom-Up

This method is a technology used in the production of nanostructured materials in which nanoparticles are formed from atoms and molecules; that is, upgrading from the original structural elements to nanoscale dimensions (Masala & Seshadri, 2004). Different types of synthesis processes are applied to bottom-up method such as chemical vapor deposition (CVD), sol-gel, hydrothermal/solvothermal, etc.

2.2.1. Chemical Vapor Deposition (CVD)

CVD depends on deposition of gaseous precursors on the solid substrate at optimum temperature contacting gaseous molecules with the substrate. There is a wide variety of CVD methods, depending on how chemical reactions are initiated and process conditions. The precursors used (metal chlorides and organometallic complexes) are compounds with high vapor pressure at low temperatures (100-400 ^{0}C). The main condition for producing high quality films with this method is high accuracy in gas velocity and precursor evaporation rate control. Chemical vapor deposition is the method that enables coatings of various structures on complex surfaces, including very curved shapes (single-crystalline, epitaxial, amorphous, polycrystalline). The

chemical vapor deposition method is highly effective for fabricating weak-aggregated nano powders of various compounds (Carlsson & Martin, 2010).

2.2.2. Self-Assembly

Self-assembly is a process by which ordered supramolecular structures are formed. The components of the original structure are virtually unchanged but are interconnected to form the resulting complex structure. Self-assembly is a concept derived from the observation of natural biological processes. In self-assembly nanofabrication, complex new materials are created with nanoscale precision through 'bottom-up' processes; therefore, it is a very important tool in nanotechnology. To obtain nanostructures, bottom-up presses starting from the bottom, such as atomic building blocks, are generally preferred instead of top-down processes. Self-assembly forms the basis of many supramolecular chemistries.

2.2.3. Sol-Gel Process

The sol-gel process is a wet-chemical technique; A gel is produced using a chemical solution or colloidal particles in a liquid medium. In the process, a stable sol containing solid particles in the solution is first prepared and subsequently gelled by a polycondensation or polyesterification reaction. The gel is dried to remove the liquid phase. In the final step, high temperature is applied for the densification and decomposition of the gels. This method is very popular technique and employed to produce metal oxide nanoparticles. Sol-gel is two-stage; sol and gel. Sol is a colloidal suspension of solid particles in a liquid

phase, while gel is the interconnected network formed between phases. The sol-gel process involves two basic reactions: (1) hydrolysis and (2) condensation (or polycondensation).

3. CHARACTERIZATION OF NANOMATERIALS

The fundamental of nanoscience lies on seeing nanoscale objects when their size is reduced to nano size. Various microscopy techniques and some spectroscopic and diffraction methods give very important information about the nanoscale structure of material. Nanomaterials are characterized by these parameters: particle size, size distribution, surface area, surface topography and surface chemistry (Kumar & Kumbhat, 2016a).

Scanning electron microscopy (SEM) is an electron microscopy method that uses a focussed beam of electrons to scan the topography of a surface and acquire the surface element distribution pattern. A focussed beam of electrons is used to scan the sample surface, which interacts with the atoms in the sample and produces different signals regarding the surface topography and composition. The detector utilized to create a picture is determined by the electron beam's location on the sample. With SEM, you can get a resolution of more than 10 nm. Studies may be carried out in a variety of settings, including high vacuum, low vacuum, wet environments, and a wide temperature range. The detection of secondary electrons generated by atoms stimulated by electron beams is the most often used scanning electron microscope mode. The number of secondary electrons identified is determined on the topography of the sample.

Transmission electron microscopy (TEM) is a microscopy technique in which a beam of electrons passing through a sample is used to create an enlarged image or a diffraction pattern. The TEM technique is generally used with specimens thinner than 200 nm. The thinner the sample, the higher the required electron beam acceleration voltage. Resolution up to 0.2 nm can be achieved. In a transmission electron microscope, a high-energy electron beam (100-300 keV) is sent to a very thin transparent test sample (<200 nm) in a system held under vacuum; The electron beam, which interacts with the sample, magnified by electromagnetic lenses is sent to a fluorescent screen, a photographic film layer or a camera, and then an image is taken. TEMs yield significantly higher resolution images than light microscopes. TEM has a wide range of applications in physical, chemical, and biological sciences, cancer research, materials science, environmental pollution, and nanotechnology studies.

X-Ray Diffraction (XRD) is based on X-ray diffraction of a single crystal in a three-dimensional crystal lattice; This technique is used to solve the atomic structure of matter, such as the unit cell's area group, size and shape, and crystal symmetry group. X-ray powder diffraction investigates the structural properties of a material in powder or polycrystalline samples. The intensity of the scattered light is obtained as a function of the scattering angle. This technique allows to determine the composition of the sample, the unit cell parameters of the sample, the material texture, the size of the crystallites of the polycrystalline sample.

4. NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

Nanotechnology is a branch of science that deals with the production of structures smaller than 100 nano-meters. This technology is promising in many fields such as engineering, physics, chemistry, biology, computer science, and medicine, with the advancement of technology. Nanotechnology is a developing field where nanoscale structures and devices are produced and used all the time. This field has a huge impact on people's lives and is continuously expanding. The use of nanotechnology is on the rise, especially in biotech and biomedical sciences. Nanoscale materials are also a growing contribution to the health of humans and have the potential to revolutionize diagnostic and therapeutic application.

Traditional bioanalytical techniques have lower sensitivity, selectivity, and efficiency than high-quality nanomaterials with well-controlled size and shape for monitoring molecular signals in biological systems and live organisms. Nanomaterials can also detect biochemical changes, allowing them to be used in low-cost, point-of-care diagnostic systems for pathogenic and hereditary disorders (e.g. HIV and cancer).

Nanomaterials are also employed in clinical imaging technologies such as MRI, computed tomography, and ultrasound as enhanced contrast agents. Nanomaterials have opened up new possibilities in biological research and clinical nanotechnology applications as a result of their unique features (Cao, 2008).

Some of the applications of nanomaterials in biology and medicine include drug and gene delivery, biological detection of pathogens and proteins, DNA structure investigation, tissue engineering, tumor destruction by heating (hyperthermia), separation and purification of biological molecules and cells, and MRI contrast enhancement (Salata, 2004).

Because of their unique qualities, such as high biodegradability and effective endocytosis with the target cell, liposomes, nanogels, micelles, and dendrimers, among others, are the building blocks of NPs. Because of their high water solubility, NPs can be employed as a carrier for cancer treatment medicines that are targeted to tumor locations. Therapeutic medicines such as small molecule medications, aptamer arrays, and antigenic proteins are being tested using NPs. Multifunctional NPs can be employed to map malignant cells as optical imaging agents, detecting probes, and other targeted biomolecules (Malhotra & Ali, 2018).

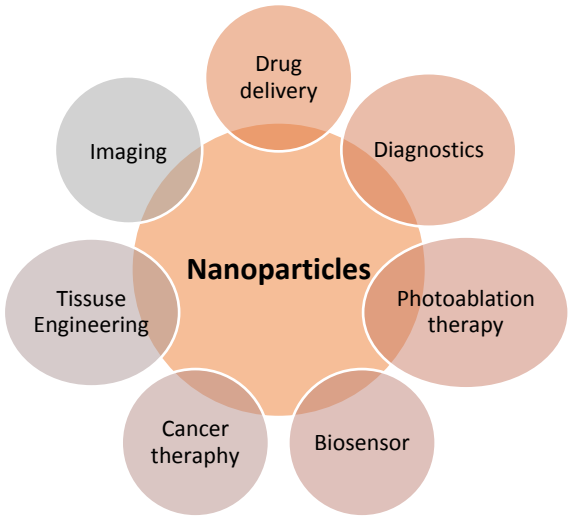


Figure 3. Nanoparticles are employed in a wide range of applications and have a multifunctional role in therapeutics, diagnostics, imaging, drug delivery, and tissue engineering (Bharathala & Sharma, 2019).

The biomedical applications where nanoparticles are frequently used are listed below and the usage areas of each application are mentioned.

- Disease monitoring
- Drug delivery
- Imaging
- Biosensing and biosensor
- Photoablation therapy

4.1. Disease Monitoring

One of the key study issues in bioengineering and medical technology is disease monitoring, which is aimed at developing diagnostic

systems to screen for complicated diseases. Diagnostic procedures are critical for detecting the origins of illnesses such as cancer, cardiovascular disease, and neurodegenerative disease, as well as monitoring the disease's improvement or progression. Today, thanks to nanotechnology, nanomaterials offer advanced diagnostic techniques. Numerous benefits of nanomaterials over traditional diagnostics, intracellular tagging, and viewing of target cells/tissues have been established. Nanotechnology has also made it possible to see tissues, cells, DNA, and proteins more clearly in a point-of-care device (Savaliya R, 2015).

These materials are mostly employed as biomolecule markers because they exhibit optical features that make them useful for a variety of diagnostic procedures, such as PCR, biochip fabrication, and multiplexing, and they are simple to use in common clinical applications. A wide range of nanomaterials, including magnetic nanoparticles, gold nanoparticles, metallic nanoparticles, quantum dots (QDs), and silica nanoparticles, are frequently employed in disease monitoring applications thanks to enhanced monitoring methodologies (Lyberopoulou, Efstathopoulos, & Gazouli, 2016).

Magnetic nanoparticles (MNPs) are gaining popularity in the biomedical field for a variety of applications, including cancer diagnosis and therapy, as well as improving tissue engineering procedures. Figure 4 shows us the functionalization and therapeutic applications of Magnetic nanoparticles (Williams, 2017).

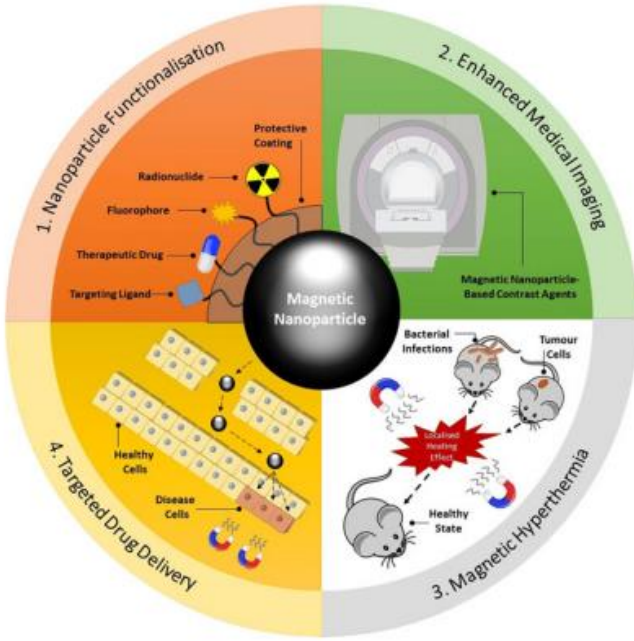


Figure 4. The functionalization and therapeutic applications of Magnetic nanoparticles.

Different disease parameters, foreign proteins/antigens, and poisonous compounds are all detected by today's nano-sized sensors. Recently, bio-barcodes for protein disease indicators such as PSA have been established (prostate-specific antigen). Based on anti-PSA antibodies, such biosensors can detect prostate cancer at an early stage. In comparison to standard approaches, the sensitivity of the test, which employs bio-barcode for prostate cancer diagnosis, is also quite high (El-Sayed & Kamel, 2020).

Nanoshells are nanoparticles that can be utilized as skin cancer scanners because of their optical, chemical, and physical features, which can discriminate between malignant and benign skin cancers.

Tumor markers comprising specific proteins are found on the outer surfaces of cancer cells and are indicative of cancer cells. The invention of nanosensor chips with antibodies specific to these indicators has resulted in a cancer detection tool that is both early and sensitive. The use of gold nanoparticles as a bio-barcode test is a significant method for detecting prostate cancer quickly (El-Sayed & Kamel, 2020).

Inorganic fluorescent nanoparticles such as quantum dots or nanoparticle-like nanophosphors, which are semiconductor nanoparticles, are used in the field of basic research in cell biology as well as in clinical diagnostic tests. Quantum dots are particularly useful as markers in image-guided techniques (Lyberopoulou et al., 2016).

The rapid diagnosis and treatment of the Covid19 virus, which has affected the whole world today, is very important for our health. Because this virus has many negative effects on the human body. Figure 5 shows us a test that makes rapid diagnosis of Covid-19 disease.

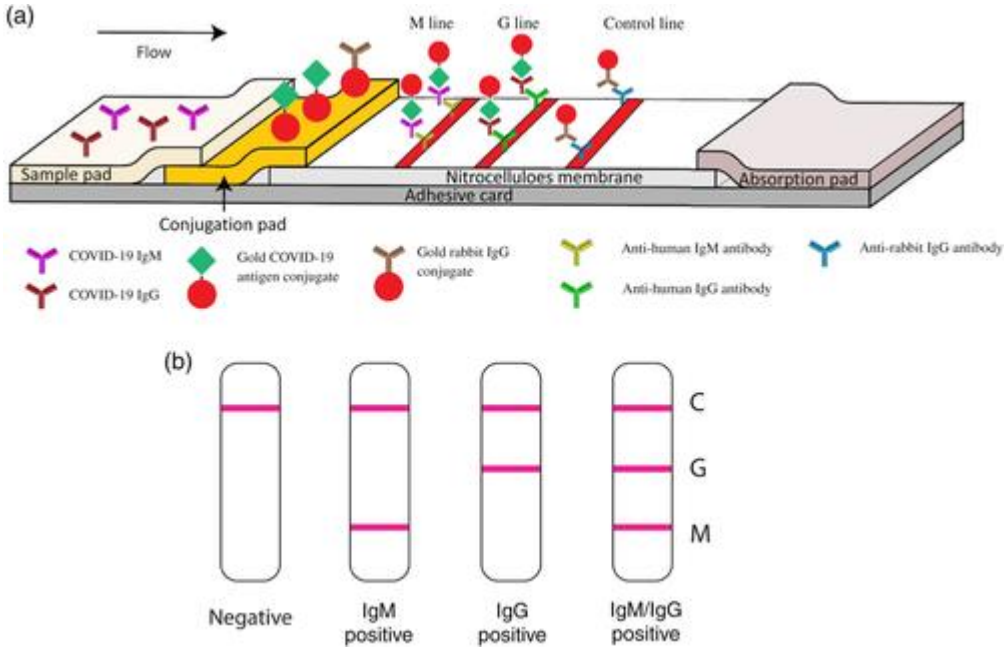


Figure 5. Schematic illustration of a test for rapid diagnosis of SARS-CoV-2 IgM-IgG combined antibody. (a) Schematic diagram of the sensing device. (b) A representation of the different test results. C: control line; G: IgG line; M: IgM line (Jianxin Wang et al., 2021).

4.2. Drug Delivery

The distribution of a pharmaceutical substance to its target site, as well as formulations, production procedures, storage systems, and technologies, is referred to as a drug delivery system [10]. Medication delivery seeks to change a drug's pharmacokinetics and specificity by including new excipients, drug carriers, and medical devices into the formulation [12, 13]. To enhance treatment results, more attention is placed on enhancing a drug's bioavailability and duration of action (A. P. Singh, Biswas, Shukla, & Maiti, 2019).

The main method for developing an effective drug delivery agent is to make a nanoparticle that carries the anticancer medicine to the proper target without causing resistance, breaking down the drug, or targeting non-cancerous cells. The medicine might be disseminated throughout the particles or contained within an aqueous or lipid-based reservoir with a polymeric protective barrier. The permeability of the tumor vasculature is exploited to boost the efficacy of drug-loaded nanoparticles (S. M. Moghimi, 2001). Nanoparticles with unique properties are readily taken up by specific recognition for drug delivery methods (Yuan, 1998). In addition, nanoparticles are frequently used in this method due to their increased permeability and retention effect (EPR) (Khan, Sakharkar, Nayak, Kishore, & Khan, 2018).

Anti-cancer drug delivery systems based on multifunctional nanoparticles have cleared the path for new therapies that are more effective, less intrusive, and less harmful (Bharali & Mousa, 2010). Nanoparticle carriers have a number of benefits over traditional treatment approaches. Because nanoparticles are more water soluble, they can be utilized as carriers for insoluble medications, removing the requirement for harmful organic solvents and their associated side effects. Using environmental (pH) or external inputs, nanocarriers may be created to control release kinetics (ultrasound, heat). This benefit of controlled release prevents the drug from being separated from the nanoshell before it reaches the tumor site, limiting drug accumulation in other healthy tissues and organs and, as a result, drug-induced systemic toxicity (Srinivasan, Rajabi, & Mousa, 2015).

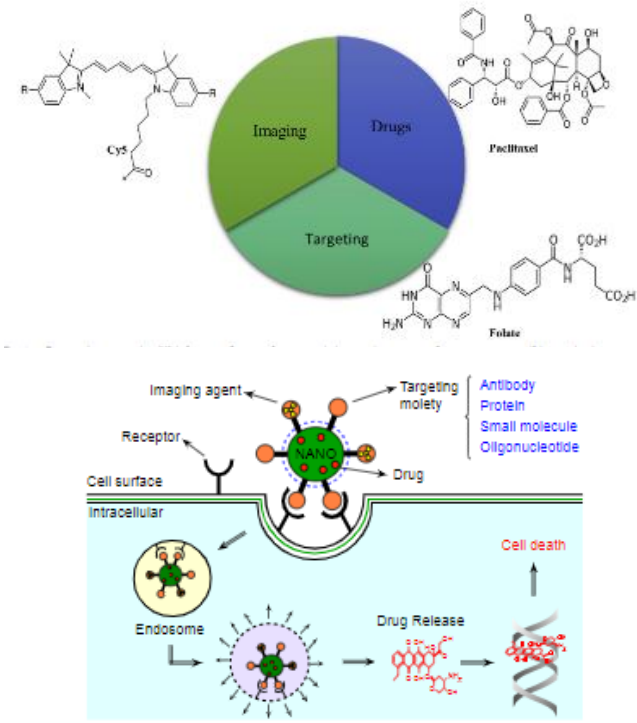


Figure 6. A schematic depiction of the method of action of a targeted multifunctional nanoparticle [5, 19].

In the drug delivery system shown in Figure 6, the cancer cell-specific ligand directs the encapsulating NP of a chemotherapeutic drug to the cancer cell surface. NP then binds to the cancer cell surface by identifying the receptor, resulting in endocytosis and internalization of NP. Inside the cancer cell, NP undergoes endosomal escape and leads to the release of its cytotoxic drug. As a result of this treatment, cell death occurs [5, 19].

Nano-sized carriers can be used to actively target tumors. Chemotherapy medications are cytotoxic, meaning they destroy

cancer cells that are actively proliferating, but they can also harm healthy cells that are dividing. Because of their surface area/volume ratio and chemistry, NPs can selectively attach to their targets on cancer cells. Internalization of the NP leads in increased drug/active ingredient cellular uptake and anti-tumor action. Targeted delivery confines the medicine's activity to cancer cells that express the desired molecule, lowering toxicity and pharmacological side effects (Yu, Park, & Jon, 2012).

Liposomes were the first nanoparticles to be employed in medication delivery. Polymeric NPs laden with chemicals have evolved to disguise their surface from proliferating immune cells, following liposomes. Polymeric NPs thwarted immune identification and removal in this way. Nanoparticles are used in drug delivery systems to target particular receptors on cancer cells with complimentary ligands linked to their surfaces, allowing them to target malignant cells with greater precision (Khan et al., 2018).

AuNPs are an efficient nanocarrier in drug delivery systems because of their unique optical and physicochemical features, biocompatibility, functional flexibility, adjustable monolayers, controlled dispersion, large surface area to load the concentration of pharmaceuticals, stability, and non-toxicity [21, 22]. Peptides, proteins, plasmid DNAs, tiny interfering RNAs, and chemotherapeutic medicines can all be transported using these efficient nanocarriers. In addition to spherical nanoparticles, stable colloidal gold nanorods have recently been investigated as a potential drug delivery method. Gold nanolattices are

another key agent. Cancer cell receptors are bound to the nanocage surface and coupled with bioactive molecules such as antibodies for targeted medication delivery (Elahi, Kamali, & Baghersad, 2018).

4.3. Imaging

Medical imaging technology is critical for the early identification of a range of diseases as well as monitoring medication responses. Today's imaging modalities include X-ray radiography, computed tomography, magnetic resonance imaging, ultrasound, positron emission tomography, single-photon emission computed tomography, and fluorescence imaging is given in Figure 7. (Ryvolova et al., 2012). To achieve more exact findings, multiple imaging modalities are frequently combined [25, 26]. In addition, contrast agents are used in medical imaging to obtain more accurate anatomical and functional information and to distinguish between normal tissue and abnormal lesions. Currently used medical imaging contrast agents are mostly small molecules that exhibit rapid metabolism and have nonspecific distribution and potential undesirable toxicities (Petrik, Weigel, Kirsch, & Hosten, 2005).

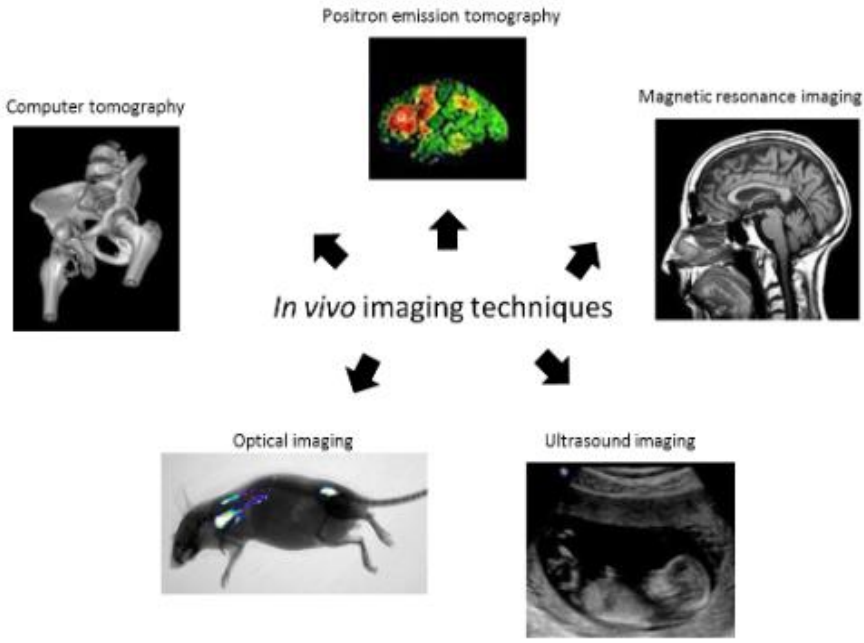


Figure 7. Main in vivo imaging techniques (Ryvolova et al., 2012).

Because of their unique passive, active, and physical targeting capabilities, nanomaterials have recently sparked attempts to enhance biological sensing and imaging. Nanoparticles have improved permeability and retention (EPR) effects in tumors due to their tiny size, resulting in relative increases in local tumor concentrations of contrast agents (Oh et al., 2013). The size of a nanoparticle is one of the most essential characteristics in tumor imaging. Biodistribution, circulatory half-life, cellular absorption, tumor penetration, and targeting are all influenced by nanoparticle size (Hoshyar, Gray, Han, & Bao, 2016). Nanoimaging agents have a higher surface area/volume ratio than traditional contrast agents, enabling for surface labeling with particular molecules and ligands to optimize toxicity and imaging

features. In addition, nanoparticles aid in functional visualization and biological imaging. Furthermore, nanoparticles may be altered to improve the loading of imaging chemicals, as well as their intrinsic physical characteristics, to fulfill specific therapeutic demands (Han, Xu, Taratula, & Farsad, 2019).

By binding on other functional molecules such as fluorescence probes or radionuclides, magnetic NPs, a popular nanoparticle, are employed as a platform to build dual-modal imaging probes. Neuroblastoma cancer cells are detected using MRI and subcellular fluorescence imaging with fluorescent dye-doped silica (DySiO2)-encapsulated magnetic NPs. Magnetic nanoparticles have also been coupled with radionuclides to create MRI-PET probes that can identify lymph node metastases in patients with high accuracy (Srinivasan et al., 2015).

The fluorescence quenching effectiveness of gold nanoparticles (AuNP) ranging in size from a few orders to hundreds of nanometers is quite high. AuNPs also offer additional benefits, including photostability, biocompatibility, varied sizes, high light scattering, and simplicity of surface labeling. Because AuNP has no renal or osmotic damage potential, a high X-ray coefficient attenuation increases contrast resolution (Zhu et al., 2017). Regardless of the changes induced by varied forms and sizes, AuNP may offer steady X-ray attenuation (Jackson, Periasamy, Bansal, & Geso, 2011). AuNP can be utilized to identify tumor cells and monitor tumor development under X-ray by acting as a tracer through cellular uptake (Han et al., 2019).

Because of their high X-ray absorption coefficient, simplicity of synthetic modification, non-toxicity, surface functionalization for colloidal stability, and targeted distribution, AuNPs have received interest as an x-ray contrast agent. Due to the longer period of vascular retention of AuNPs compared to regularly employed conventional agents, the creation of a viewing window is the outcome of their features (Elahi et al., 2018).

AgNPs may be employed in a wide range of biomedical techniques, including imaging and therapies, because to their unique physiochemical characteristics, with antimicrobial agents being one of the most important uses. Surface plasmons are also supported because of their distinctive optical characteristics. Quantum dots are commonly utilized nanoparticles for in vitro and in vivo imaging and cell labeling. These nanoparticles have also been studied extensively as biological imaging agents, and they have emerged as a new class of fluorophores with near-ideal properties such as high quantum efficiency, a broad excitation spectrum, a narrow, tunable, and symmetrical emission spectrum, and ease of bioconjugation (S. K. Singh, Kulkarni, & Dash, 2013).

4.4. Biosensor Applications

Biosensors are electronic devices that combine biological sensing material with a chemical or physical transducer to transform a signal into a quantifiable and accessible electrical signal. Biosensors are frequently used in a wide variety of fields, from medicine to food. Figure 8 depicts the general mechanism of action of a biosensor. A

biosensing substance must be present in biosensors, and this biosensing substance must recognize the chemical or analyte of importance. In addition, the biosensing substance must be in near vicinity to a transducer, which can electrically translate and detect incoming signals (Li Yanbin, 2006). Various compounds, which are enzymes, antibodies, and whole-cell, are used to catch particular biomolecules (Mishra, Sharma, & Mishra, 2018). Biosensors are categorised as optical, thermometric, electrochemical, or piezoelectric depending on the kind of transducer used for data processing and assessment (Akyilmaz & Yorganci, 2008).

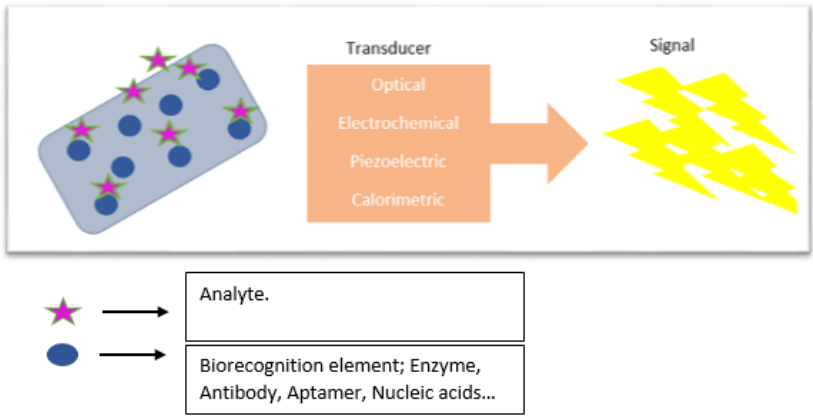
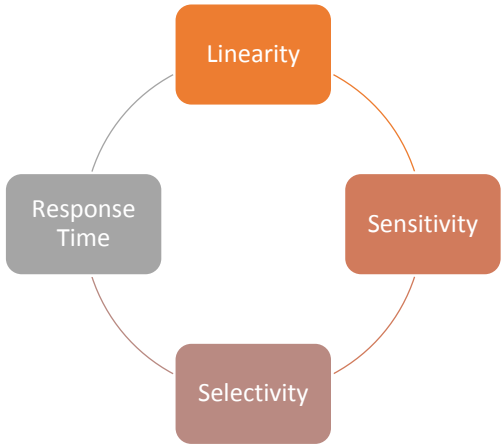


Figure 8. The basic structure of a biosensor.

Some of the most important features of biosensors are listed below.



- ✓ Due to the usage of bioreceptors, biosensors have a distinct set of characteristics.
- ✓ Biosensors have higher sensitivity and specificity than conventional sensors. However, because of their sensitivity to the working environment, they may be lacking in robustness.
- ✓ Biosensors may suffer from stability or performance deterioration over time since they are made up of biological components; for example, enzymes or antibodies may lose their function over time. Operating life is influenced by storage conditions and production procedures.
- ✓ Biosensors are frequently used at point-of-care settings.
- ✓ Biosensors frequently have a restricted working range in terms of temperature, pH, and humidity in which they will dependably operate.
- ✓ Biosensors are difficult systems because they require previous sample preparation.

- ✓ The time necessary to get 95% of the responses should be minimal.
- ✓ The sensor response per analyte/substrate concentration should have a high value.
- ✓ To get the best result, chemical interactions should be kept to a minimum (Agnese Magnani, n.d.).

The advantages and disadvantages of biosensors are also indicated in Table 1 (Malhotra & Ali, 2018).

Advantages	Disadvantages
Ease of use	Quality of results
Transportable	Clinic oriented operators
Not processed samples	Inappropriate and excessive use
Quick results	Price
Small sample volume	Conformity with regulations

Today, nanotechnology is used everywhere. Detection techniques have improved considerably since the introduction of different nanomaterials and their unique properties. The application of various nanomaterials like gold nanoparticles, magnetic nanoparticles, quantum dots, and carbon nanotubes to biosensors has resulted in significant advancements in biomolecule identification. The use of nanomaterials in biosensors that can diagnose various diseases has a very important role in sensitivity and selectivity. Thanks to the modification of nanomaterials with biosensors, groundbreaking

methods will be developed for early detection and intervention (X. Zhang, Guo, & Cui, 2009).

Because of their tiny size, nanoparticles possess unique features (Perumal & Hashim, 2014). Biosensors have shown tremendous potential for the detection of chemicals and biomarkers since nanomaterials are used as signal transducer components to indicate the identification of the analyte (Antiochia, 2021). The strong adsorption and catalytic activity of nanoparticles are attributed to the presence of a significant number of active sites and functional groups on their surface (Q. Zhang, Wu, Xu, Ma, & Zhang, 2021).As a result, nanomaterials may be used to create sophisticated and specialized detection systems such as electrochemical sensors and biosensors. Some of the most common include nanomaterials AuNPs, multi-walled carbon nanotubes (MWCNTs), and graphene (Cavalcante et al., 2021).

- **Gold Nanoparticles in Biosensor Application**

Due to their favorable optical, chemical, and catalytic capabilities, gold nanoparticles are among the most common metallic nanoparticles used in biomedicine. Gold nanoparticles, which are known to be inert and oxidation resistant, may be made in a variety of ways, with varying effects in terms of particle structure and shape. The surface of these nanoparticles, like that of many other nanoparticles, may be changed with diverse moieties such as antibodies, small molecules, and peptides, allowing for specific targeting by physical adsorption or covalent bonding. Furthermore, gold nanoparticles may be

programmed to respond to changes in the external environment, such as pH, temperature, brightness, certain frequencies, electricity, magnetic stimulation, electric current, and even the application of external heat. Stimulus-sensitive nanoparticles are intended to respond to a variety of internal and external events in order to deliver medications to the target region (Freire, Fechine, Neto, & Nascimento, 2021).

Many sensors have been developed for the purpose of detecting gold nanoparticles and their varied features. Gold nanoparticle sensors can be colorimetric, fluorescent, electrical and electrochemical, surface plasmon resonance, surface-enhanced Raman scattering (SERS) based, quartz crystal microbalance based, or Bio-Barcode test sensors, depending on the detection approach (Yeo et al., 2017).

Immunomagnetic separation is used to collect and concentrate influenza viruses, as shown in Figure 9. Fetuin-AuNPs are then incubated with MNP-Virus to create a sandwich of MNP-Influenza virus-AuNPs, which is then trapped using a permanent magnetic field. Finally, the quantification of AuNPs is used to conduct indirect chronoamperometric detection of viruses (Mollarasouli, Zor, Ozcelikay, & Ozkan, 2021).

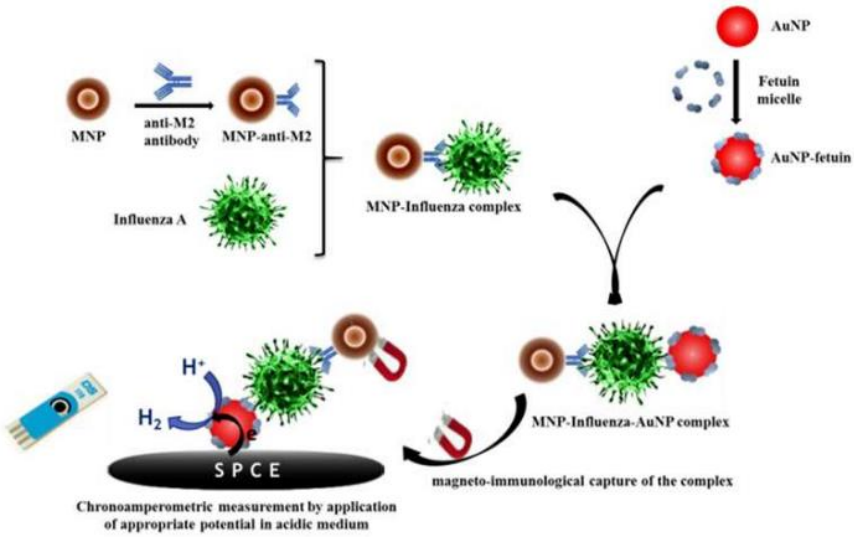


Figure 9. Schematic depiction of to construct the gold nanoparticle-based chronoamperometric magneto-immunosensor for the influenza virus (Mollarasouli et al., 2021).

❖ Magnetic Nanoparticles in Biosensor Application

Catalysis and biotechnology/biomedicine applications are both interested in magnetic nanoparticles with good stability. Catalysts, nuclear waste, biological products, and organisms can all benefit from magnetic nanoparticles like these. Magnetic separation can be utilized in biotechnology and biomedicine as a quick and straightforward way to capture certain proteins or other biomolecules efficiently and reliably (Lu, Salabas, & Schüth, 2007).

The ability to concentrate the analyte before detection is a significant benefit of utilizing magnetic nanoparticles in biosensor systems. Magnetic nanoparticles are also a viable replacement for fluorescent labels in biosensor devices. Because there are less magnetic fields in

nanosized magnetic nanoparticles than in bulk material, they have a distinct magnetic behavior called superparamagnetic behavior. This implies that the magnetization can change direction at random in a very short amount of time, and so appears to be at mean zero in the absence of an external magnetic field. This is a temperature-dependent phenomena that may be prevented by aligning the magnetic moments with an external magnetic field. Bioanalytical applications are where iron oxide is most commonly used (Holzinger, Goff, & Cosnier, 2014).

4.5. Photoablation therapy

Photodynamic therapy and photothermal therapy are the two forms of photoablation treatments. Non-toxic photosensitive compounds called as photosensitizers are used in photodynamic therapy, which become dangerous when exposed to light of a specified wavelength.

This treatment is most commonly used to treat damaged cells, such as those seen in cancer. Photo-induced electrons and holes are generated when photosensitizers, such as TiO2 nanoparticles, are subjected to a certain wavelength of light. Reactive oxygen species (ROS) and singlet oxygen are formed when photo-induced electrons and holes mix with hydroxyl ions or water. As a result of the development of these species, cell death is inescapable. Photothermal therapy, on the other hand, uses a near-infrared (NIR) light source to irradiate tumor cells. Hyperthermia and cell death can happen from the conversion of light energy into heat energy. TiO_2 offers a variety of appealing features, including biocompatibility, chemical stability, and

photocatalytic activity. It is because of these characteristics, notably its photocatalytic activity, that it is a desirable species for use in photothermal treatment [48–51].

Figure 8 depicts the photocatalytic mechanism and reaction for TiO2. Excitation, diffusion, and surface transfer are the three phases in the photocatalytic process for TiO2. The nanoparticles absorb photons from a light source in the first stage. This energy is enough to push the electron across the band gap and into the conduction band. The valence band develops gaps as a result of this process. The holes and electrons are subsequently dispersed onto the photocatalyst's surface. The formation of chemical reactions at the surface is the final stage in this photocatalytic process. These reactions are triggered by the formation of holes and electrons (McNamara & Tofail, 2017).

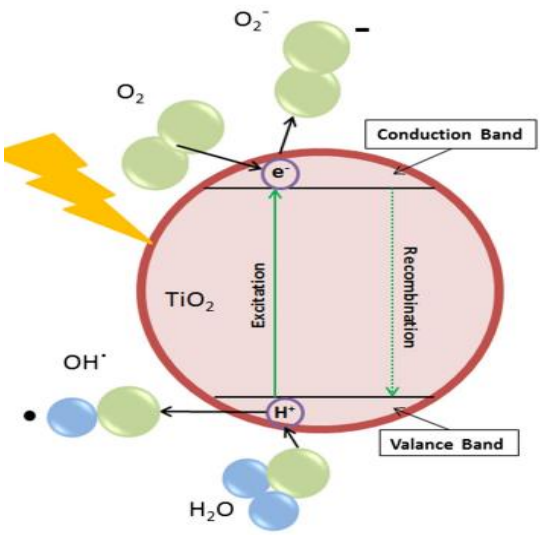


Figure 10. Schematic showing the photocatalytic process of TiO_2 nanoparticles. (McNamara & Tofail, 2017).

AuNPs utilized in photodynamic treatment have key features such as effective fluorescence quenching and surface plasmon resonance (SPR) absorption. Furthermore, due to its affinity for thiols, disulfides, and amines, gold conjugation increases intracellular penetration (Narang, Malhotra, Singh, & Pundir, 2015). AuNPs with greatest absorption in the visible or near-IR range absorb light and create heat in photothermal treatment. Malignant tumors are killed by heat [52, 53]. Furthermore, AuNPs-antibody conjugates can be used for diagnostics as well as photothermal treatment (Elahi et al., 2018).

When the particle size is significantly smaller than the incoming wavelength, the liberated electrons cannot traverse over the surface as they do in conventional surface plasmon resonance (SPR) settings due to the unique optical features of gold nanoparticles. The electron density is thus polarized on one side of the particle, where the plasmons oscillate with light in frequency. This phenomena, which is significantly dependant on the size and shape of the nanoparticle as well as the dielectric constant of its surroundings, was described using the Mie theory. This environmental dependency provides a substantial benefit for bioanalytical since the recognition event may induce a variation in the oscillation frequency and hence a color shift of the gold nanoparticles that can be observed with the naked eye. A wide range of efficient colorimetric biosensors for DNA or oligonucleotide detection, as well as immunosensors, has been developed in this respect (Holzinger et al., 2014).

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BÖLÜM 5

DETECTING SNP MARKERS USING ASSOCIATION MAPPING ANALYSIS THROUGH GENOTYPING-BY-SEQUENCING (GBS) METHOD IN COTTON

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INTRODUCTION

Cotton (*Gossypium spp*.) plant has remerkable importance economically, and grown in arid and semi-arid regions as source of naturel fiber uses in textile, and oilseed. Alone, it supplies 35% of the fiber used in the world. Gossypium genus contains six tetraploid (At Dt)1 to (A t D t)5, where the "t" indicates the allotetraploid, "A" and "D" represents the genomic groups (Grover et al.; 2008). There is about 50 species of cotton, and 45 of them are known to be diploid (2n = 2x = 26), and 5 of them are allotetraploid (2n = 4x = 52) (Zhang et al., 2014). Diploid species (2n = 2x = 26) are G. arboreum L. and G. herbaceum L. and clasified into 8 genomic group (A-G and K) (Cao., 2015). Tetraploids species (2n = 4x = 52 are G. hirsutum L. and G. barbadense L. with genome group (AADD) (Hui-fang, 2013).

Approximately 100 million families cultivate cotton from 35 countries in 33.5 million hectares worldwide (ICAC., 2017/2018; FAO, 2020;). The cotton plant provides raw materials to the industry with 17-24% ratio of oilseed and its fibers contain 94-96% of cellulose and constitutes the main source of livelihood for approximately 180 million people worlwide (Akçar., 1986). The textile industry, oil industry, ginning industry, feed industry, and paper industry get benefit from its cotton. As an alternative to petroleum, the oil obtained from the cotton kernel is used in biodiesel production. It provides raw materials to almost 50 branches of industry (Anonymous., 2020).

Developing new high yielding resistant varieties against various biotic and abiotic factors which decrease cotton yield and quality, grown in

regions with arid and semi-arid climates, has become an important field of study for cotton researchers. Yield, disease, and pest resistance are complex traits that are controlled by multiple genes (Polygenic)(Crowel et al., 2016). However, studies on resistance in plants showed that resistance is characterized not only by the genotype (genetic), but also by the environmental impact (non-genetic) (Jäger et al., 2014). The determination of the genotypic difference (DNA sequence) underlying the phenotypic variation has been made possible by developing DNA markers, and polymorphic DNA markers have been identified by identifying the polymorphism in DNA (Myles et al., 2009).

Molecular quantitative developments in genetics allow the researcher to choose a genotype in MAS using a molecular marker that is tightly linked to the gene/genes that control the desired trait (Zhao et al., 2017). The method which uses markers in F_2 and next generation for genomic is called Marker-assisted selection (MAS) (Smith and Simpson., 1986). MAS is also known as molecular plant breeding, or marker-supported breeding. A DNA marker is a specific DNA fragment of a certain length. A spesific marker is tightly linked to the gene/genes responsible for the traits, and even marker-QTL in segregation process are not separated. An ideal marker should be located on Chromosome less than 5 cM (Santimorgan) from the gene or QTL. The marker is at a certain distance next to the QTLs that are responsible for the trait and represent the relevant QTL (Xu and Crouc., 2008; Ben-Ari and Lavi., 2012).

In parallel with the rapid development of the genetic markers used in QTL mapping, and Association mapping studies, the developments in genotyping technology accompanied the formation of the Association mapping map, which allows the study of complex genetic features and reveals the marker-character relationship by using the population structure, population information and kinship information between the individuals of the population (Vinod., 2011). Association mapping is used to eliminate the disadvantages of QTL mapping. It can identify the gene/genes responsible for the desired trait and which QTLs on which chromosome are located and indicate the distance between markers and QTLs as cM (centimorgan), so this mapping method is highly preferred by the molecular breeders (; Abdurakhmonov and Abdukarimov., 2008; Kraakman et al., 2004).

In the literature, Association Mapping (AM) is known as association analysis, association studies, and linkage disequilibrium mapping. AM shows the response of polymorphism in DNA sequences in phenotype; therefore, it can establish the link between loci in the genotype to the phenotype (Chakraborty and Weiss., 1988). In other words, AM aims to determine the link between the phenotype and the genotype (Botstein and Risch., 2003). Association mapping determines the quantitative trait loci (QTL) related to the desired traits. The difference from QTL mapping is the populations types. The purpose of both is to identify polymorphic DNA markers associated with the trait of interest.

AM eliminates the disadvantages of Linkage Mapping or QTL Mapping (Family based mapping, genetic, gene, genome mapping, gene tagging) (Mackay and Powell, 2007) which is using hybrid populations such as backcrosses (BC), second filial generation (F_2), Recombinant Inbred line (RIL), Double haploid (Stich and Melchinger., 2010). At the same time, AM analysis is a QTL determination mapping study that uses natural populations such as non-hybridization, Landraces populations, breeding varieties, and materials based on the non-random relationship alleles at different loci (linkage disequilibrium). Thus, with AM, it is possible to identify Single Nucleotide Polymorphism (SNP), and is able to explain the genotype-phenotype relationship of individuals (Jannink et al., 2010; Zhu et al., 2008).

Similarities And Differences Between Association Mapping (AM) And QTL Mapping

QTL mapping has synonym names in the literature, such as genome mapping, gene tagging, and gene mapping, while AM is known as Linkage disequilibrium mapping. AM-based linkage disequilibrium (LD) is an alternative when QTL mapping is not available (Ross-Ibarra *et al.*, 2007).

The purpose of both QTL mapping and AM is to identify genes (QTLs) that are responsible for any trait. Although both are based on the principle of LD between the molecular marker and the loci.

The association mapping idea called LD is known as the non-random association of alleles at different loci and firstly was introduced by

Jennings in 1917. It was developed by Lavontin (D) or D'(corrected D), which is a measure of LD (Abdurakhmonov and Abdukarimov., 2008). Another measure of LD is "r^2". The r^2 measure is the square of the coefficient of the relationship between the two loci (Hill and Robertson., 1968).

While the r^2 measure reflects different aspects of LD, it shows different performances under various conditions, while D reflects recombination only and preferred statistical measure for expressing recombination differences (Flint-Garcia et al., 2003). r^2 is intensely preferred in determining LD for providing more information about marker and QTLs, and it is preferred that this coefficient be between 0.1 and 0.2. (Gupta et al., 2005). These values are accepted as the minimum threshold of the important relationship between two different loci pairs and indicate the physical and genetic distance where LD is significant (Zhu et al., 2008).

Abdurakhmonov et al. (2008) did association mapping analysis of cotton and reported that the average LD value throughout the whole genome in the local cotton varieties was 10 cM at r2 ≥0.1, and 30 cM in other varieties. Abdurakhmonov et al. (2009) stated that LD at r^2 ≥0.1 reduces the genetic distance of 50 cM and that LD of r^2 ≥0.2 reduces the ability of association analysis by 5-6 cM. Witt and Buckler (2003) stated that LD decreases to 25 cM genetic distance in r^2 ≥0.1. As the value of r^2 approaches “1” and the p-value approaches “0.0001”, the importance of the marker increases, and it gains great

importance in the point of being the QTL of the searched marker (Flint-Garcia et al., 2003).

1. ASSOCIATION MAPPING (AM) ANALYSIS

In general, AM analysis takes place in 5 stages (Fig 1). **Stage 1**: Obtaining germplasm (Genetic stock materials) with different genetic properties. **Stage 2**: Repetition cultivation of germplasm materials in different environments and years and determine properties such as stress tolerance, yield quality, and phenotypic data. **Stage 3**: Screening of all genotypes with Molecular markers such as SSR, SNP, AFLP, SRAP (Genotyping data). **Stage 4**: Calculation of population structure, kinship, LD, and its measurements such as D, D 'or r^2. **Stage 5**: The phenotyping data of the marker data obtained in genotyping (GLM and MLM) analyzes should be performed, and results should be obtained (Al-maskri et al. 2012).

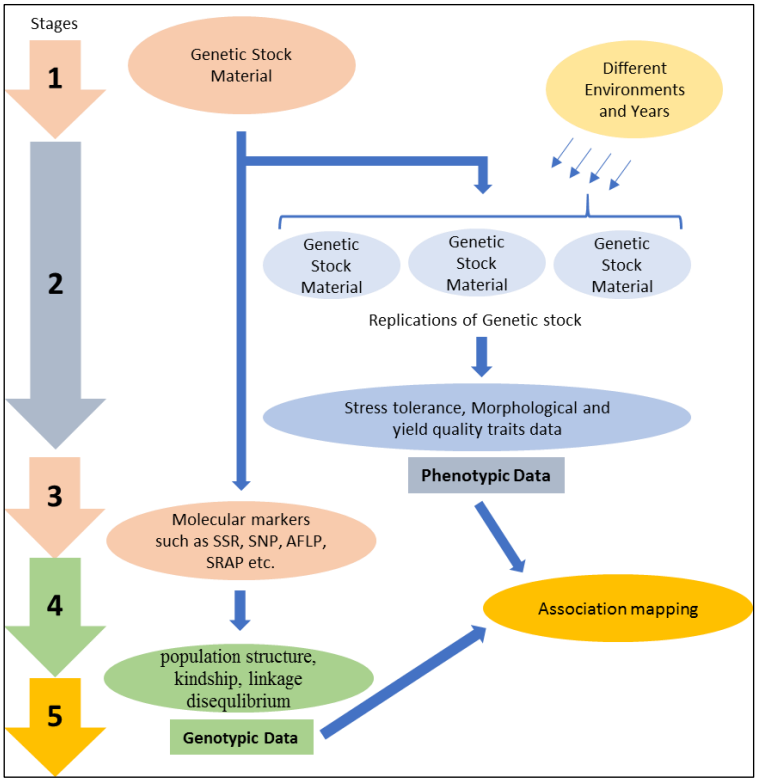

Figure 1. Association mapping analysis general procedure (Al-maskri et al. 2012).

1. 1.Phenotyping Analysis

Field trials should be established at different locations (at least two location) to obtain more more reliable datas in different years. However, since the field is uncontrolled, establishing an experiment in the climate room under controlled conditions will increase the study's effectiveness. Phenotypic data will be taken according to the parameters that measure the desired trait, and variance analysis of the phenotypic variations will be performed with such the JMP 7.0, SAS (Statistical Analysis Software), IBM SPSS 25.0 version, and so on… statistical analysis software.

1.2. Genotyping Analysis

1.2.1. Genotyping-by-Sequencing (GBS)

DNA extraction kit or CTAB (Cetyl trimethylammonium bromide) DNA isolation protocol, developed by Zhang and Stewart (2000), can be used for genomic DNA isolation. The isolated DNA will run in 50 (mA) electrical power as electrophoresis in 1% agarose gel in a TAE (Tris-acetate-EDTA) buffer [(1XTAE; 4.84 g Tris, 1.142 ml Acetic Acid, 2 ml 0.5 Molar (M) EDTA (Tris-Acetate-EDTA, Ph: 8)] and display under ultraviolet (UV) light. DNA concentrations will be determined with Nanodrop Spectrophotometer (Thermofisher), and DNA samples of the genotypes will be genotyped by GBS (Genotyping-by-sequencing) method (Fig 2). At the end of GBS analysis, Single Nucleotide Polymorphism (SNP) raw data will be obtained. GBS analyzes will be performed according to the method proposed by Elshire et al. (2011).

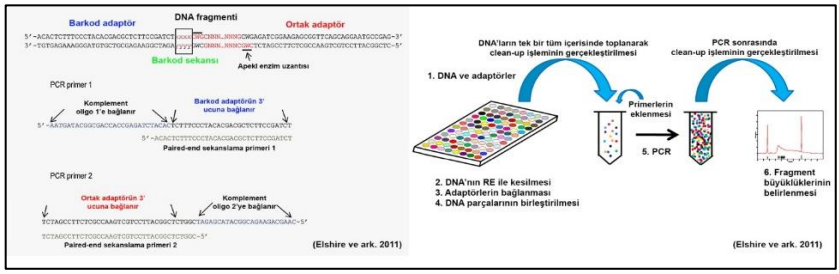

Figure 2. Application of GBS Method (Elshire et al. 2011).

GBS method uses the Illumina MiSeq, and the HiSeq2500 platform allows for a large amount of SNP for genetic analysis (Bentley et al., 2008) (Beissinger et al. 2013).

GBS analysis, which cheaply provides a lot of SNPs and short time, has staged such as cutting genomic DNA with restriction enzymes, creating a library, sequencing, raw data, obtaining high-quality data, SNP determination, and genetic mapping as a result of clustering and sequencing analysis of these data. GBS is an application of Next-generation sequencing (NGS). This platform display DNA bases from 100 million to 100 trillion in a cycle (He et al., 2014).

1.2.2. Obtaining Marker Data (hmp. Extension)

Raw SNP data will be uploaded in MS Excel will be saved as Text (Tab delimited). Then starting TASSEL 3> Load> I will make my best guess and try> Selecting the text file and run. Click on Data> sequence> file name and Export. "Write HapMap" is checked and choose the file type to export the dialog box. The data obtained are called "HapMap" marker data or genotyping data with "hmp" extension to be used in TASSEL 5.2.10 computer software version (https://www.maizegenetics.net/tassel).

1.2.3. Populatıon Structure And Kinship

Bayesian model-based (MBB) clustering software Structure 2.3.4 version calculates (Pritchar et al., 2010) the population genetic structure (Q matrix). Program settings to determine the genetic structure (K) of its population; the permutation module of Markov Chain Monte Carlo is selected as 10,000-100,000, the K value is between 1-15, and 10 repetitions are made for each K value. K values give the optimum values of the cluster (Pritchard ve ark., 2000). The results are Zip. The file is uploaded to the web-based analysis method

“Structure harvester” software to calculate and determine the best Delta K value. Matrix values in ΔK are used in association analysis. As a result of the Kinship calculation, the negative values among relatives are accepted as "0".

1.2.4. Linkage Disequlibrium (LD) Analysis

LD in the literature is also known as gametic disequilibrium (GD), gametic phase disequilibrium (GPD) or allelic association, is based on the non-random relationship of alleles among non-related individuals who make up a population according to Hardy-Weinberg law (Jannink and Walsh., 2002). LD can provide information for the marker density and intensive high-resolution mapping required in the Association mapping studies (Gupta et al., 2005). LD has parameters such as D, D ', r^2, D_2, D *, F, and δ are generally used in mapping analysis (Gupta et al., 2005; Devlin and Risch., 1995). However, in most of the studies, it was observed that D 'and r^2 ‘were used extensively as LD measures (Gupta et al., 2005). Linkage equilibrium and Linkage disequilibrium mathematical calculation is as follows;

Linkage disequlibrium; PAB ≠ PA x PB; Linkage equlibrium, PAB= PA x PB

A and B are alleles at two different loci, and PAB is the haplotype frequency at two different loci. PA and PB only show haplotype frequencies of the A and B alleles (Gupta et al., 2005). The LD analysis is based on the calculation of the square (r^2) of the coefficient of the relationship between all marker pairs and is calculated by the program TASSEL 5.2.10 (Trait Analysis by aSSociation, Evolution,

and Linkage) (Bradbury et al., 2007). With the TASSEL 5.2.10 version program, P-value was calculated for each r^2 value with Fisher's bidirectional test (Zhao et al. 2014). The minor allele frequency (MAF) of the SNP markers (TASSEL 5.2.10> Filter> Sites) obtained by the GBS method is less than 0.05 (MAF <0.05), LD is calculated with the software TASSEL 5.2.10.

1.2.5. Marker-Trait Association Analysis (Association Mapping)

In AM analysis, factors such as Population bottlenecks, founder effects and drift which reduction in population size, the use of a small number of individuals, and low frequency of specific alleles can determine false positive association (Gupta et al., 2005). Mixed linear model (MLM) analysis is performed using Population structure and kindship to eliminate such type of error (Type 1 error) (Yu et al., 2006). TASSEL software provides opportunities to determine the relationship between DNA markers and traits (Bradbury ve ark., 2007). The General Linear Model (GLM) and the Mixed Linear Model (GLM) identify the marker responsible for the trait by providing marker-trait matching. In Association mapping analysis, parameters such as p-values are between P <0.0001 and p <0.05; the r^2> 0.1, and LOD score> 3 increases the probability of the marker to be the marker is looking for (To perform Association mapping analysis in TASSEL 5 versions the highest version of Java should be set up in computer).

1.2.6. General Linear Model (GLM)

Association mapping studies were performed according to the general linear model (GLM) (Pritchard et al. 2000) and mixed linear model (MLM) (Yu et al., 2006) methods. Phenotypic, Q-matrix, and genotypic data are used in the GLM method in association analysis. The purpose of this analysis is to determine the relationship between marker-traits.

1.2.7. Mıxed Liner Model (MLM)

In the MLM method, Q-matrix, kinship, phenotypic and genotypic data is used. In addition to the Q-matrix (Structure), which gives information about the structure of the population, the use of K-matrix (kinship coefficient between Germplasm individuals), which gives information about the degree of kinship, has led to increase the efficiency and reliability of the MLM method (Raman et al., 2010). MLM method is known to be used for control purposes to arrange mismatches (Pritchard et al., 2000). P-value and r^2 are calculated for each marker-trait association with the MLM analysis method (Bradbury et al., 2007).

According to the statistical formula defined by Henderson, 1975, MLM analysis is done using the Q + K parameters specified below.

$$y=X\beta + Zu + e$$

"y"; n X 1 vector of observation, "X"; unknown n X p matrix, "β"; unknown vector, "Z"; known vector n X q, "u" ve "e" are random vectors that cannot be observed.

The traits data and the Structure program's data are combined in a common Excel file and saved in Text (Tap delimited) format. Then the marker data obtain from TASSEL 3 software with hmp. extension and will be combined with the trait data to perform the analysis in TASSEL 5.2.10 software. This analysis and getting the results to take place in 10 steps. **First step**: Starting TASSEL 5.2.10 >Data menu>Load>Make best quess of dialog box> marker (hmp.) and trait data; **Second step**: Selection marker data>Filter>Sites>Minimum Freuquency 0.005, maximum frequency 1 and miminum case 1 >fill. Remove Minor SNP states and then filter; **Third step**: Selection filtered data>Analysis menu>Linkage disegqulibrium>Full matrix; **Fourth step**: Selection filtered data and trait data>data menu>Intersect joint> finally combined both marker and trait data; **Fifth step**: Selection combined data>Analysis menu>GLM analysis>run permutation with 1000 number; **Sixth step**: Selection filtered data>Analysis menu>Kindship>Scaled-IBS; **Seventh step**: Selection kinhsip and combined data>Analysis menu>MLM>Filtred out put on P-value; **Eighth step:** Select LD marker data>Results menu>LD plot> save; **Nineth step**: Selection GLM stats>Results menu>Manhatton plot>Select trait and save, **the Tenth step**: Selectin MLM stats >Results menu>Select trait>save as. To get the data obtained as a result of GLM and MLM analysis, MLM and GLM analysis results are selected and exported in excel format by saving the table from the Results menu. The data exported in Excel format are sorted according to the smallest P and r^2 values.

CONCLUSION

The breeding method that uses DNA markers saves time and labor and provides safer, realistic results and requires less professionalism, and is not affected by environmental conditions. To develop resistant varieties against biotic and abiotic factors and it is required to know which genotypes carry the desired traits. Millions of SNPs (Single Nucleotide Polymorphism) can be determined simultaneously with the GBS method. Such as low cost, realization in a short time, and SNP determination throughout the genome advantages led to intensive use in AM analysis. AM reveals the marker-trait association based on LD and determines the QTL that controls the relevant polygenic trait. After validation, QTLs will be used as markers in the F_2 (MAS) stage of hybridization. The MAS method, which eliminates the disadvantages of classical breeding, provides such an opportunity to screen with molecular markers whole population individuals in the initial stages of variety development to determine whether the desired trait is available in new varieties candidates.

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BÖLÜM 6

BIOTECHNOLOGY APPLICATIONS OF BORON DERIVATIVES

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1. INTRODUCTION

Boron is the chemical element with atomic number 5 and is symbolized with the letter B. Boron is a semi-metal. It is a low amount element both in the Solar System and in the Earth's crust. However, due to the water solubility of its compounds (borate minerals) found in nature, it can be found in high concentrations in certain places. Boron can react with various metal or nonmetal elements to form more than 200 different compounds with different properties. However, elemental boron cannot be found in nature.

Among about 230 kinds of natural boron minerals in nature, especially those with commercial value; tincal, colemanite, ulexite, probertite, borasite, pandermite, szyabelite, hydroboracite and kernite.

Boron is one of the indispensable elements for the survival of all living things in nature. Besides, Boron is one of the most common elements in the world.

There are many studies showing that boron is an element that must be taken during the growth and development of living things. In particular, boron, which has proven to be necessary for the cell wall in plant development, is an important element in maintaining the health of the plant. Since it is not produced by the human body, it is an element that must be taken from the outside (Hunt, 2012). It can enter the body by oral route (food, drink and vitamins), by inhalation or by absorption through the skin (soap, detergent and cosmetics). The majority of orally ingested boron is rapidly converted to boric acid in

the gastrointestinal tract and the majority is excreted within 24 hours. Boron compounds are used in different fields such as nuclear, defense industry and metallurgy, as well as in the health sector. With recent studies, boron compounds have started to give effective results by using them in many treatments (Nielsen, 1997). Boron and its esters, which are preferred in fields such as tissue engineering and biomaterials, regenerative medicine, pharmacology, are preferred in the production of drugs with their antioxidant activity and in cancer treatment, in tissue engineering with mineral and vitamin regulation, in burn treatment and wound healing process with increasing vascularization. In addition, boron compounds have an important place in the treatment of osteoporosis and arthritis, thanks to the acceleration of the bone development process.

1.1. BORON

Boron, whose chemical symbol is shown with B in the periodic table, has atomic number 5 and atomic weight of 10.81, is a semiconductor element between metal and nonmetal. Boron element is located in group 3A of the periodic table and consists of two different stable isotopes called B^{10} and B^{11} in nature. The rate of occurrence of B^{10} isotope in nature is 19.1%-20.3%, and B^{11} isotope is 79.7-80.9%. The element boron, which is never found in free form in nature, forms compounds with different properties with various metal or nonmetal elements. Due to this feature, more than 200 different boron compounds are formed and give different chemical, physical and physicochemical properties to the products it enters. With these very

different features it brings to the products, it is known as the salt of the industry and can be used in more than 250 areas of the industry.

Boron is an element that does not exist in nature. Because boron forms compounds with carbon and other elements, high pure boron is uncommon in industry. Boron comes in a variety of forms: amorphous boron is a dark brown powder; crystalline boron is black, exceedingly hard (approximately 9.5 on the Mohs scale), and low conductivity at room temperature. In the semiconductor business, boron is employed as a dopant. It is the most significant component of MgB2 technology, which is a high-purity elemental boron superconductor.

Boron minerals are found as hydrate compounds with calcium, sodium and magnesium elements and are classified according to these elements. Boron minerals with commercial value; Tincal, Colemanite, Ulexite, Probertite, Boracite, Pandermite, Szaybelite, Hydroboracite and Kernite. After the boron mines are extracted from the soil, they can be washed, broken or ready for use.

In addition, Refinery boron products are; Borax Pentahydrate (Refined, Calcined), Borax Decahydrate (Refined, Calcined), Boric Acid, Anhydrous Borax, Synthetic, Refined Colemanite, Calcined, Calcined Hydroboracite, Calcined Ulexite and Calcined Tincal.

Also, boron is a strong electrophilic compound and Lewis acid because it contains an empty p-orbital. It can easily bond with nucleophiles and thus convert the uncharged trivalent planar structure to the anionic tetravalent structure. This feature of the pipe to him; can

interact with nucleophiles such as hydroxyl and amine groups found in enzymes, carbohydrates and nucleic acids.

1.2. BORON DERIVATIVES

Natural Boron Compounds

From boron's high affinity for oxygen; There are a wide variety of boron-oxygen compounds in nature, the simplest ones being boron oxide (B_2O_3) and boric acid, and their general names are known as borate. In addition, boron can easily form compounds with elements such as sodium, magnesium and calcium. The most known of them are; Colemanite ($Ca_2B_6O_{11}.5H_2O$), Tincal ($Na_2B_4O_7.10H_2O$), Borax (($Na_2B_4O_7.10H_2O$)) and Ulexite ($NaCaB_5O_9.8H_2O$). These compounds are also known as natural borates.

Almost all boron ores produced around the world are either marketed in chunks or ground concentrate and used following an enrichment operation. Such products can be classified as raw boron.

The most common borate in humans and animals is boric acid, also known as hydrogen borate. Since it is easily soluble in water, it is quickly metabolized and excreted in a very short time after being taken into the body. Boric acid is a colorless, odorless and easily soluble crystalline and lewis acid compound. In humans, the respiratory tract epithelium, mucosal membranes of gastrointestinal tissues, mouth, vagina, and anus absorb 90% of the injected amount of boron and distribute it equally throughout the body (Cui et al., 2004)(Bakirdere, Orenay, & Korkmaz, 2010)

Boric acid and other borates react with sugar moieties to form sugar borate esters-(chelates). The first sugar-borate compound obtained from plants in nature is Calcium-Fructoborate. Today, CaFB is chemically synthesized and commercially marketed as a food supplement.

Synthetic Boron Compounds

Due to their unusual reactivity and other appealing properties, organoboron compounds (Boron containing compounds-BCCs) have provided the foundation for the discovery of a growing number of new and novel reactions that have already been used in many areas of synthetic chemistry, from pharmaceuticals to materials (petasis). For a long time, many features of their chemical characteristics and reactivity have been understood. Boron-containing compounds: chemico-biological characteristics and therapeutic promise in prevention, diagnostics, and treatment (Soriano-Ursúa, Das, & Trujillo-Ferrara, 2014).

The study, design, and synthesis of novel organoborons for medical benefit has risen in popularity due to certain chemical characteristics of boron. The ability to adopt tricoordinated-SP2 or tetracoordinated-SP3 geometric configurations, the ability to form covalent bonds with carbon, nitrogen, or oxygen, the function as isosteres of boron-free acids, and the effect on the isoelectronic nature of different chemical interactions most commonly found in biological systems (e.g., C=C and B-N bonding) are just a few of these properties (Das et al., 2013).

The resultant organoboranes have become one of the most commonly used reagents and intermediates in organic synthesis, including asymmetric processes, since the discovery and development of hydroboration. Furthermore, early study into the chemistry of borohydrides and carboranes has uncovered new families of compounds with distinct structures and reactivity that continue to pique interest.

Despite these early findings, the actual promise of some of the chemically stable and versatile organoboron compounds was not realized until recently. Organoboronic acids, boronates, and the recently found organotrifluoroborate are now among the important organoboron compounds, in addition to organoboranes. Many of these compounds have found use in synthetic chemistry, asymmetric synthesis, metal-catalyzed reactions, and acid catalysis.

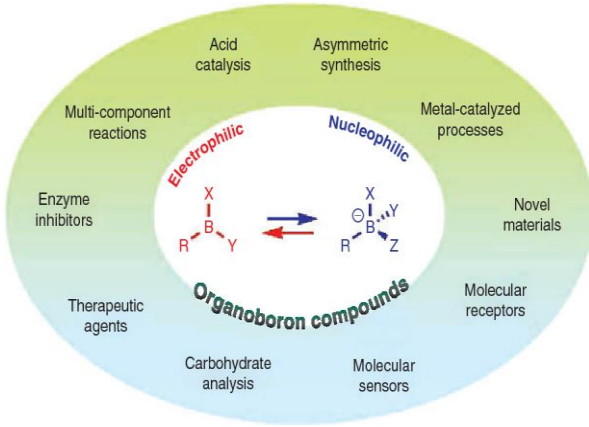

Figure 1. Chemical applications of organoboron compounds (Petasis, 2007)(Petasis N.A).

Boron was first discovered from the Maesa icta seed in 1857, and its toxicity was first described between 1800 and 1900. In 1910, the physiological importance of boron was discovered and it was determined that it is an essential element for the growth and development of many plants. In 1923, it was defined that boron is one of the essential micronutrients for plant growth and development, and that boron has physiological importance. These data revealed the idea whether boron is also important in animals, but although positive results were not obtained in the studies carried out on boron by nutritionists in the 1950s; In 1980, Nielsen et al.'s research on chickens also revealed that boron is important in animal development (Hunt, 1994). Boron, which was defined as a trace element in the 1980s, was classified as a possible basic element in terms of human health by the World Health Organization (WHO/WHO) in 1996. The necessity of boron for humans and animals has not yet been demonstrated, but it is a fact that it has beneficial effects. It is claimed that boron, which is reported to be an essential trace element for the cell, has important functions in mineral metabolism, lipid metabolism and energy metabolism, immune and endocrine system as well as the brain, positively affects performance, and may be effective in the prevention of osteoporosis, osteoarthritis and arthritis (Nielsen, 2008)(YESİLBAĞ, 2008) In addition, in clinical trials, it has been determined that the probability of developing prostate cancer in men is significantly reduced when boron is taken with foods (Cui et al., 2004). However, the molecular mechanisms of boron's function in animals are not yet understood (Tanaka & Fujiwara, 2008).

Boron is not produced in living things, it is taken from food, drinking water and some of the products they consume (cosmetics, detergents, soaps, etc.) by being exposed to boron. The main source of boron taken from food was determined to be fruits with about 35%. The amount of boron taken with natural foods varies according to people's eating habits and gender. Natural boron compounds taken from food; Known as sugar-borate, it is found in vegetables, fruits, seeds and nuts.

The daily boron intake determined by WHO is expressed as 1-13 mg for adults. Besides the acceptable safe range for boron in food, the upper tolerable dose level is specified as 20 mg/day. The dose level without any adverse effects (NOAEL) is 9.6 mg/day, while the lowest observable adverse effect dose level (LOEL) is 13.3 mg/day (Caroli et al., 1994)(Prejac et al., 2018).

Boron can be used in its raw form, or it can be converted into refined boron compounds, turned into end products and in all three forms; It is widely used in many fields of industry, especially in the glass and detergent industries. In addition, it has a wide range of uses ranging from fertilizers and pesticides to flame-resistant materials, electronics and space technologies, agriculture and livestock sector (Das et al., 2013)(Velioğlu, SAYLI, & Altunsoy, 1999). While, it is used in agriculture as an insecticide, herbicide, micronutrient, it finds use in ceramics, nuclear reactors, photography, plastic, textile industries, fire extinguishers, adhesives, wafer production, make-up materials, electrical insulation and disinfectants (Velioğlu et al., 1999).

In the field of health, boron compounds are used in the treatment of osteoporosis and rheumatoid arthritis, in the treatment of brain tumors with Boron Neutron Capture Therapy (BNCT), in burn treatments, wound healing, antiseptic in lens solutions, ointments, biological growth regulators (as a food supplement). appears to be used in mouthwashes and eye drops (Doonan & Lower, 1978)(Kuru & Yarat, 2017).

Boron is believed to be involved in a variety of physiological and metabolic processes in microbiological systems, plants, and animals [11]. However, like other chemicals, it is known to have a hazardous impact on organisms when exposed to excessive amounts. According to research, boron is involved in nucleic acid metabolism, carbohydrate and protein metabolism, cell wall production and construction, and membrane integrity protection in plants (Moseman, 1994). The requirement for boron during embryological development in animals was first observed in zebrafish and trout embryos (Miwa et al., 2007)(Ruby I Rowe, Bouzan, Nabili, & Eckhert, 1998). However, the molecular mechanisms of the effects of boron on animals are not yet understood (Ruby Imagene Rowe & Eckhert, 1999). Although its necessity for humans has not yet been proven, it is a fact that it has beneficial effects. It is reported that it affects cell membranes, plays a role in steroid hormone metabolism and is necessary for healthy bone development. In addition, in clinical trials, it has been determined that the probability of developing prostate cancer in men is significantly

reduced when boron is taken with foods (Tanaka & Fujiwara, 2008)(Cui et al., 2004).

Boron has been researched in various distinct forms, including boronic acid, boron nitride, borax, and fructoborate, in the international literature and studies on health.

Today, boron-containing synthetic organic compounds find widespread use in synthetic organic chemistry. Recently, boronic acid-containing polymers have been used in various biomedical applications; For example, it has been proven that it can be used in vital diseases such as HIV, obesity, diabetes and cancer treatment.

2. BORON ON BIOTECHNOLOGY APPLICATIONS

Polymers provide a number of well-known benefits in biotechnology, particularly drug delivery, such as enhanced activity due to multivalency and the capacity to release drugs gradually and controllably with tailored biodistribution. Macromolecular drug delivery vehicles have a longer circulation period in the body because their relatively large size decreases glomerular filtration rates (Seymour, 1989).

The reticuloendothelial system (RES) can readily clear small molecules from the body, whereas large hydrophilic polymer-based systems may not be as easily detected, resulting in a prolonged circulation period. Due to their unique reactivity and stimuli-responsive nature, boronic acid-containing polymers have potential applications as self-healing materials, therapeutic agents, self-

regulated drug delivery systems, nucleotide adsorbents, and sugar and glycoprotein sensors (Cambre & Sumerlin, 2011).

2.1. Lipase inhibition

Prior to absorption by the digestive tract, dietary fat must be hydrolyzed. Lipases are enzymes that hydrolyze lipids that are insoluble and hydrophobic. Lipases break down lipids so that they can be absorbed by the digestive system. Lipases and proteases have been found to be inhibited by phenylboronic acid groups.

Boronic acid inhibition is thought to occur when trigonal boronic acid forms a negatively charged tetrahedral combination with a serine hydroxyl group in the lipase active center. The enzymes have a 102-104 times higher affinity for boronic acid residues than for typical lipid substrates. Lipase inhibitors operate by inhibiting lipid breakdown in the digestive system, decreasing fat absorption. Undigested triglycerides and diglycerides are then eliminated from the body without being absorbed (Cambre & Sumerlin, 2011).

2.2. HIV İNhibition

The HIV virus causes AIDS (acquired immune deficiency syndrome). In 2009, an estimated 2.6 million people become HIV positive for the first time, with 1.8 million of those living in Sub-Saharan Africa. While HIV may be transmitted in a variety of ways, unprotected heterosexual intercourse is the most prevalent form of transmission. Given this, it is evident that women-controlled prophylactics or microbicides are required to prevent HIV infection. Understanding

vaginal physiology and the mechanism of heterosexua HIV transmission is required for the development of efficient microbicides.

Precoital vaginal fluid has an acidic pH, ranging from 4-5. Sperm can neutralize vaginal fluid due to its alkaline composition, high buffering capacity, and larger volume. The virions can then infect CD4 T-cells, macrophages, and dendritic cells by passing past the vaginal epithelium and into the subepithelial region. Active compounds in microbicides target the mucosa, tissue, or cell/virus surfaces, as well as interrupting the replication cycle within the cell.

The hydrogels produced by phenylboronic acid moieties and diols had a responsive viscoelastic behavior with enough fluidity for application, but when the pH was raised after insemination, they converted into a strongly crosslinked network that entrapped HIV-1 virions and prevented mucosal penetration. This physical barrier has the potential to prevent HIV infection in its infancy.

Microbicides for HIV also function by blocking the virus from entering cells. The gp120 envelope proteins are mostly responsible for HIV-1 entry into CD4 cells. The benzoboroxole sites on the polymer might theoretically react with the mannose residues on gp120, rendering HIV-1 inactive before it reaches CD4 cells and preventing HIV transmission (Danial, Root, & Klok, 2012).

2.3. Controlled drug release and delivery

Drug delivery refers to a variety of methods for delivering a pharmaceutical chemical into the human body in order to accomplish

and/or maximize the desired therapeutic effect(s) while minimizing any negative consequences. Chemicals, peptides, antibodies, and vaccines, as well as gene-based medications, are examples of pharmaceutical molecules. Based on the route of administration, drug delivery systems can be divided into several groups. Novel drug delivery methods such as targeted delivery and drug-device combinations are currently garnering more and more interest in drug research, in addition to classic techniques such as oral, injectable, transdermal, inhalation, implant, suppository, ophthalmic, and otic dosage forms (Wen, Jung, & Li, 2015).

Boron nitrides (BN) are structural counterparts of carbon (C) materials with alternating B and N atoms replacing C. Although further research is needed, BN materials, such as boron nitride nanotubes (BNNTs), have been demonstrated to have superior biocompatibility and lower cytotoxicity than their C counterparts. As a result, designing and exploring biomaterials based on the BN system should be a viable and promising option. Boron Nitrides, which are highly water-soluble, porous, and biocompatible, are utilized to deliver anticancer drugs (Weng et al., 2014).

2.4. Biosensing Application with Boron Darivatives

Boron derivatives with electroactive and fluorescent properties, such as boron doped hybride polymers, boron doped nanostructures, and boron doped electrod materials, have been employed in biosensing systems. Carbon nanotubes are more thermally and chemically stable than boron nitride nanotubes (BNNTs) (CNTs). BNNT is a viable

contender for applications such as biosensors because of these features, as well as its outstanding mechanical and thermal conductivity (Panchal & Upadhyay, 2014).

Boron-doped diamond (BDD) thin films are attracting a lot of attention as a new type of electrode material. The advancement of diamond growth by chemical vapor deposition has made it possible to create BDD electrodes with a variety of surface structures on a variety of substrates. The BDD electrodes' electrochemical characteristics have also been thoroughly investigated (Yu, Zhou, Wu, & Zhi, 2012). BDD electrodes were shown to have a wide electrochemical potential window, a low and stable capacitive background current, great response repeatability and long-term response stability, and good biocompatibility, according to the findings. BDD electrodes are now frequently employed in the creation of electrochemical biosensors as a result of this.

Saccharide sensing with boronic acid

In the last decade, there has been a resurgence of interest in main group organometallic chemistry, as well as a slew of surprising findings. The creation of hybrid polymers, which combine main group elements with conventional organic structures in one framework, is an attractive subject in terms of novel materials with unexpected features. When a sugar binds to a boronic acid moiety, optical property changes or conductivity changes are commonly used in saccharide sensing with boronic acid-based systems (Benderdour, Bui-Van, Dicko, & Belleville, 1998). Because of their capacity to covalently bind diols

(sugars) and boronic acid-containing polymers It can be used to treat diabetes in particular.

2.5. Boron Neutron Capture Therapy (BNCT)

The synthesized boron molecules are organoboron molecules. Molecules with single borons are usually aryl boronic acids. Multiple boron-containing BNCT agents, typically polyhedral borane anions or carboranes, are much more effective. These agents are organoboron synthesis studies and their importance is increasing day by day.

BNCT is a form of radiotherapy that is used especially in the treatment of neck and aggressive brain tumors. This treatment has become a preferred treatment for reasons such as selective destruction of sick cells, minimal damage to healthy cells, and the potential to be the ideal treatment for cancer types that are difficult to surgically treat (such as brain cancer). BNCT has been a known treatment for nearly 60 years. The main goal of this treatment using boron; It is made by neutron bombardment of the drug form of boron, p-bronophenylalanine and its derivatives, after they are administered to the tumor tissue. This method is based on the fact that Boron settles in the cells and after neutron bombardment, only tumor cells are selectively destroyed. Thus, since the cell whose DNA is fragmented cannot enter the division cycle, the tumor tissue can thus be treated with BNCT without the need for surgery and without damaging the healthy cells. In summary, the physical basis of BNCT is quite simple, a binary system based on nuclear reaction. To the stable isotope of

boron (^{10}B), when low-energy or thermal neutrons are irradiated (bombarded with a neutron accelerator); Helium-4 (4He) (ie α particle) and Lithium-7 (7Li) nuclei are formed. These high-energy charged particles cannot move far and leave all their energies to the tumor cell, thus preventing the reproduction of cancer cells by directly damaging the cells they are in used in the treatment of BNCT; a biotechnology laboratory where boron-containing amino acids (L)-4-dihydroxy-borylphenylalanine (BPA), sodium borocaptate (BSH).

Another cancer treatment method using boron-containing molecules is photodynamic therapy. Photodynamic therapy (PDT) is a therapy applied to kill cancer cells by using light with special drugs called photosensitizing agents (photosensitizers). These special drugs only work when activated by certain types of light. PDT is also known as photoradiation therapy, phototherapy, or photochemotherapy. PDT is a valuable treatment option, especially in the treatment of localized (not spread) cancers. Boron-dipyrromethenes (BODIPYs) are a new class of dyes that are showing excellent results as photosensitizers (PSs) in PDT.

The study and applications of these dyes increased exponentially since the early 1990s, due to their attractive properties (Benniston & Copley, 2009). Their intense fluorescence, good stability, and its chemical versatility, notoriously increased researchers interest on these compounds and its use in a large number of applications (Zhang et al., 2011).

Most BODIPY dyes are relatively easy to synthesize starting from commercial pyrroles and acid chlorides, anhydrides, aldehydes or ketones (Krumova, Greene, & Cosa, 2013). In addition, the core of BODIPY presents a great reactivity and chemical versatility that allows the incorporation of different functional groups and the generation of new compounds with diverse properties (Al-Sheikh-Ali, Cameron, Cameron, Robertson, & Thompson, 2005). Usually, BODIPY derivatives present absorption and emission bands in the visible region with high molar absorption coefficients (~ 40,000–110,000 M−1 cm−1). In addition, they have high quantum fluorescence yields, property that makes them excellent fluorophores (Benstead, Mehl, & Boyle, 2011). BODIPYs do not usually present phosphorescence processes because the excitation of these chromophores generates a low concentration of triplet states by intersystem crossing (ISC).

In addition, BODIPY dyes are used as an alternative method in the photodynamic inactivation (PDI) of microorganisms in the treatment of infections caused by antibiotic-resistant microorganisms.

3. CONCLUSION

Boronic acid-containing polymers have lately shown useful in a number of therapeutic applications, including HIV therapy, obesity treatment, diabetes treatment, and cancer treatment. Despite their particular reactivity, solubility, and responsiveness, boronic acid-containing (co)polymers are underused in comparison to many other groups of functional polymers. This chapter focuses on research in

this field, with a special emphasis on recent advances in synthesis, processing, and materials development that have enabled the production of novel biomaterials. Furthermore, throughout the previous decade, boron neutron capture therapy (BNCT) has been used for cancer treatment, when chemotherapy and radiation both have flaws.

In the future, depending on the development of synthetic chemistry, both the promising Boron-dipyrromethenes (BODIPYs) molecules used in Photodynamic therapy (PDT) and BNCT molecules; New ones will be added to the existing ones. Apart from the organoboron molecules containing boronic acid, other organoboron compounds will be added as well as the development of usage areas.

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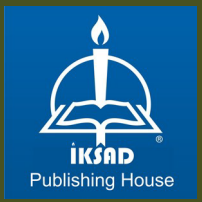

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