Chapter 1

Introduction to Food Toxicants

1.1 Food Toxicity

Food contains a range of natural compounds; all its constituents are not supplements. Some of these molecules may reduce the nutritional value or even be hazardous (e.g., naturally occurring toxicants). Similarly, synthetic substances may be accidentally or purposefully added when food is being prepared and processed [1]. Food risks can have a variety of origins, such as metabolic byproducts of plants, animals, microbes, environmental, chemical, and biological hazards, and food additives. Food additives are chemical substances added to maintain or boost-up freshness, nutritional value, safety, taste, and texture. These food additives enter the body because food is consumed as an essential item to get energy for its functioning. Not only food additives but also food contaminated with pathogenic bacteria, toxins, or environmental impurities can cause severe problems since it can result in many health issues [2]. More than 200 diseases, including typhoid, diarrhea, and other foodborne illnesses, are caused by contaminated food and can be fatal [3]. Pathogenic agents are estimated to represent 81% of sicknesses and hospitalizations and 64% of deaths because of foodborne diseases [4].

Despite foodborne illnesses, contaminated foods with pesticides, drug residues, and industrial chemicals are an essential issue that impacts public health [5]. That is why food safety plays a vital role in the quality of livelihood, physical and mental health, and illness control. The foodborne risk assessment ensures food safety through a scientifically based process, including hazard identification, hazard characterization, exposure assessment, and risk characterization. After careful risk assessment, a new quality assurance standard for food safety is developed. Hazard Analysis and Critical Control Point (HACCP) is a systematic approach used in food production to ensure food safety. Here is an overview of various food hazards, risk assessment, management, and prevention.

1.1.1 Types of Food Toxicity

Toxicity is the degree of damage to the human organs or hampers the metabolism due to physical, biological, chemical, or a mixture of these substances. These substances may be natural, synthetic, or semisynthetic. The leading cause of contamination in food mainly comes from polluted water, pharmaceutical residues, pesticide residues, chemical additives, organic pollutants, metalloids, and microplastics from marine products like salt. Based on the types of contamination, food toxicity can be classified as physical, biological, and chemical toxins [6].

(a) Physical Toxin

Physical Toxins are commonly exterior materials that are not parts of our food chain like wood pieces, small bones in meat, dust particles, broken teeth, sand, hair, etc. These materials are ordinarily nonhazardous but are associated with unhygienic surroundings such as bacteria that may cause infection. Extraneous material can be considered hazardous due to its hardness, sharpness, size, or shape. When these materials reach the human metabolism system, they cause several injuries to the metabolism system. The physical toxins are easily recognized and removed from the food. The general physical toxin is described in **Figure 1**. Washing and filtration of food will remove sand, dust, wood, insects, and other physical toxins.

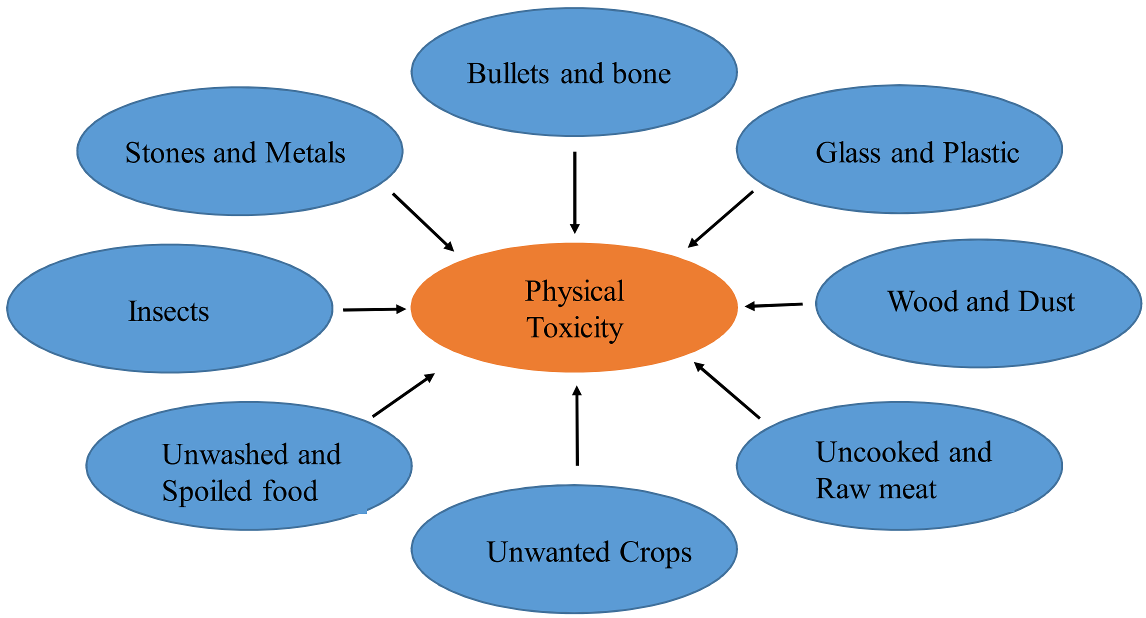

Figure 1.1: *Types of physical toxin*

(b) Biological Toxin

Biological toxins are natural substances that contain many molecules generated by Fungi, Algae, pathogens, viruses, bacteria, and genetic modification crops with harmful effects on human metabolism even at low concentrations [7]. The most common biological toxins are described in **Figure 2**. Biological toxicity may be controlled by temperature and irradiation processes. Bacteria such as Staphylococcus aureus, Salmonella enteritidis, E. coli, and Campylobacter grow on food; leaving out for a long time at room temperature can cause illness. Heat treating the food can reduce the risk of microbial spoilage and extend the product's shelf life. Food irradiation by ionizing radiation (gamma rays, electrons, or X-rays) controls foodborne pathogens. It reduces microbial load or destroys bacteria, fungi, and other parasites that cause food to get spoiled.

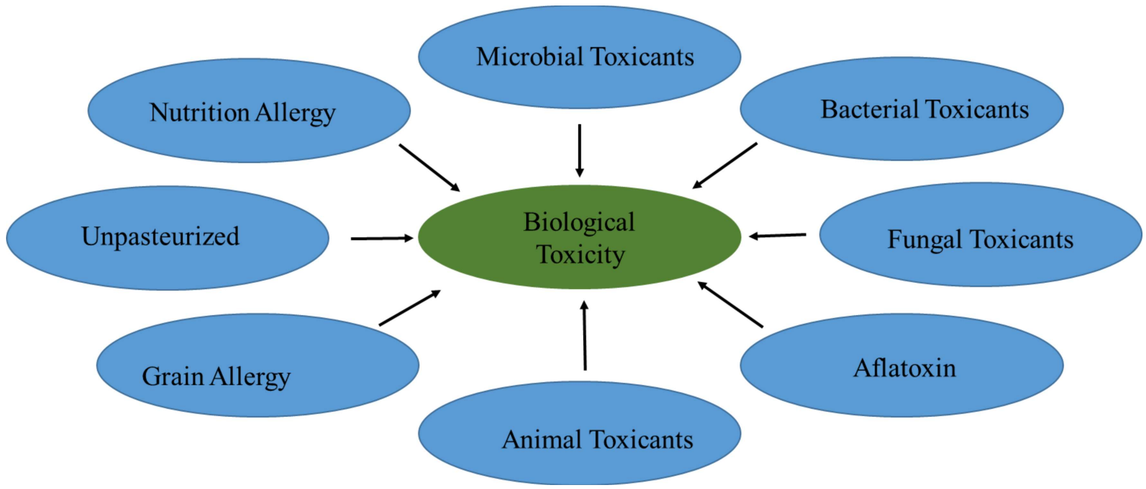

Figure 1.2: *Types of a biological toxin*

(c) Chemical Toxin

Human food consists of different types of chemical substances which may be toxic under specific circumstances [8]. These substances may include chemical contamination, drug residue, pesticides, Maillard reaction products, organic pollutants, heavy metals, metalloids, marine biotoxins, mycotoxins, insecticides, environmental contamination, water pollutant, metal ion, packing materials, and food additives [9][10]. These chemicals cause intense adverse effects on human health, such as cancer, intestinal ulcer, Parkinson's disease, Alzheimer's, and genetic disorders in long-term use. The food additive is frequently used in proceed food and is the leading cause of illness mentioned above.

Other chemical toxins can be described as part of food additives when they extend the permissible range. For example, the human diet includes thousands of different chemical compounds. A food additive is any material that, either directly or indirectly, becomes a component of or otherwise influences the properties of any food, according to the US FDA. Nutrients, colorants, sweeteners, herbicides, pesticides, and flavoring agents are a few examples of Food additives that may be either naturally occurring or purposely or inadvertently added. Natural components undergo chemical modifications when these ingredients are added to food during processing and preparation. Food additives are added to achieve specific technical features like color, sweetness, flavor, preservation, and other physicochemical effects. The FDCA recognizes three categories of food additives [11].

1. Intentionally added substances
2. Substances that are natural components of food
3. Substances that may contaminate food

Food additives have become more widespread in recent years due to the increased production of processed foods. Several food additives produce hazardous radiolytic compounds when exposed to radiation. For example, turkey ham contains 0.1 percent potassium benzoate, an antibacterial agent. When subjected to 2 kGy radiation and refrigerated for six weeks, this ham creates the volatile chemical benzene by decarboxylating potassium benzoate [12]. A similar process is used to develop volatile benzene in acidic drinks, including benzoic acid and ascorbic acid [13]. Consuming more than 1 gram of monosodium glutamate daily might result in palpitations, blisters on the arms and occasionally the anterior thorax, and general weakness and weariness [14]. The food industry uses various additives to obtain or enhance the numerous technical properties during food preparation [15]. The development of safer food items is a crucial concern for the food industry, as described in several reports[16]–[25]. In **Table 1.1**, many kinds of food additives are outlined.

Table 1.1: *Toxicity of food additives*

S. No.	Category of food additives	Application	Example	Toxic Effect Reported
1	Colorants	Colorants are used to improve physical appearance.	Erythrosine, tartrazine, and quinoline yellow,	DNA damage, Cancer, asthma, migraine, hyperactivity, headaches, etc.
2	Preservatives	Prevention from the growth of microorganisms	Sodium benzoate, potassium nitrate, and calcium benzoate	Fetal abnormalities, neurotoxicity, asthma and carcinogenic, etc.
3	Sweeteners	Use to obtain a sweet taste with calories	Aspartame, mogrol, neohesperidin, and acesulfame	Lymphoma, cancer, myeloma, leukemia, etc.
4	Antioxidants	Use to delay or prevents oxidative processes	Oregano, basil, rosemary, pepper, BHT, and propyl gallate	Joint pain, eye problems, stomach, asthma, dermatitis, etc.
5	Acidity regulators	pH regulator	Sorbic acid, benzoic acid acetic acid, citric acid and propionic acid	Dental cell toxicity, mutation aberration and chromosomal, etc.
6	Flavor enhancers	For taste enhancement	Monosodium glutamate, aspartame, arginine, saccharine,	Parkinson's disease, Chinese Restaurant Syndrome (CRS), Alzheimer's disease, Depression, Schizophrenia, and birth deformity in posterity among pregnant women

1.2 Monosodium Glutamate and its Adverse Effect

Monosodium glutamate (MSG) is an extensively used food additive as a taste enhancer in commercial foods. L-Glutamic acid is a non-essential amino acid found in its free form. This amino acid is found abundantly in many foods naturally. The chemical formula of glutamic

Table 1.2: *MSG is natural foods*

S.No.	Fruits	FGA mg/100g	Vegetables	FGA mg/100g
1	Grape	5 - 184	Spinach	48
2	Apple	4	Chinese cabbage	100
3	Kiwi	5	Corn	106
4	Grape juice	258	Green asparagus	49
5	Processed	230	Onion	51
6	Tomato	140 - 246	Green peas	106
7	Grapefruit	11.5	Potato	10 - 102
8	Avocado	18	Cabbage	50
9	Fresh tomato	260	Carrot	33
10	Peach juice	32	Shiitake mushroom	150
11	Grapefruit juice	18.6	Sweet potato	60
12	Nectarine	9.6	Shiitake mushroom	71
13	Prune (dry)	18.6	Broccoli	176
14	Orange juice	21	Mushroom	42 - 180
15	Prune	14.4	Truffles	8.5
16	Strawberry	44.4	Soybeans	66

Table 1.3: *MSG is seafood, poultry, and meat foods*

S. No.	Seafood, Poultry, and Meat	FGA mg/100g	Eggs/Dairy	FGA mg/100g
1	Cured Ham	337	Stilton	820
2	Pork	2.2 - 9	Parmesan	1200- 1680
3	Beef	10	Emmenthaler	308
4	Pork Fillet	40	Cheddar cheese	182
5	Chicken Bones	40	Gruyere de Comte	1050
6	Chicken	1.5 - 22	Roquefort	1280
7	Blue crab	43	Danish Blue	670
8	Alaska king crab	72	Gouda	460
9	Snow crab	19	Camembert	390
10	Bonito	285	Goat Milk	4
11	Scallop	140 - 159	Saint Paulin	210
12	Mackerel	215	Whole chicken egg	15
13	White shrimp	20	Cow Milk	1
14	Cod	44	Chicken egg white	0.2
15	Sardines	280	Human breast milk	19
16	Tuna	188	Chicken egg yolk	46

acid is HOOC - CH_2 - CH_2 - $CH(NH_2)$ – COOH. It is a white solid with a molecular weight of 147.13 g and a melting point of 205°C. The pK values for the ionizing groups of glutamic acid are 2.1 (R-COOH), 9.47 ($R\text{-}NH^{3+}$), and 4.07 (side chain) [26]. The chemical formula of MSG is $C_5H_8NO_4Na$, and the IUPAC name is sodium 2-aminopentanedionic. It is well-known as a glutamate and sodium salt of glutamic acid. Its molar mass is 169.11 g/mol and has a melting point of 232˚C. It naturally exists in many foods, including parmesan, tomatoes, cheese, seaweed, soy sauce, and breast milk. These are outlined in **Tables 1.2** and **1.3.** Although glutamate is flavorless when attached to dietary proteins, MSG is present in these natural foods as glutamic acid and its bound form as glutamate. Hydrolysis of dietary proteins is required for the umami taste of MSG, which is obtained from the free glutamic acid. Bounded glutamate is fragmented into smaller peptides and free amino acid forms during some food processing methods. These techniques for food preparation can raise the amount of free glutamic acid in food.

1.3 Objective of the Thesis

Food industries analyze thousands of samples daily to declare the content of MSG on the label; however, it is often difficult for the authority to keep tracking these declared quantities due to the accessibility of diagnostics systems on the site. Thus, it is necessary to have a point of a diagnostic system that is simple, fast, selective, sensitive, efficient, and cost-effective. The regular monitoring of glutamate is also required for fermentation control during its production; thus, such a system is also usable there. On the other side, street food vendors use MSG very frequently without considering the upper limit set by the Food Safety and Standards Authority of India (FSSAI).

Hence, considering the importance of MSG limit in foodstuff, in the Indian scenario, it is most important to quantify its concentration in food samples with reliability, accuracy, and low cost. MSG concentration is quantified by various techniques, like chromatography, surface plasmon resonance, chemiluminescence, spectrophotometry, fluorescence, capillary electrophoresis, and electrochemical biosensor in the lab. Different L-glutamate electrochemical biosensors based on L-glutamate oxidase and L-glutamate dehydrogenase have recently been reported. This methodology may overcome the constraints of the analytical techniques by developing it similarly to the glucometer works for testing glucose levels by the individual.

In contrast, the electrochemical methods have limitations in specificity and narrow detection range (μM) of MSG. Despite several advantages of electrochemical systems, practical usability has been restricted by the challenges of developing platforms for working electrodes, which simultaneously incorporate a more significant proportion of bioactive functional groups and show adequate charge transportation at the working electrode's surface. Hence, this thesis focuses on developing an electrochemical immunosensor for quantifying MSG to overcome the low selectivity and specificity limitations with monoclonal anti-glutamate antibodies, which provide high specificity and enhanced selectivity and affectability for electrochemical detection of MSG.

In this context, the present study has increased the detection range by synthesizing new nanocomposites of polymer-metallic nanoparticles, conductive polymer-metal oxide nanoparticles, and conductive polymer-metallic nanoparticles for developing highly conductive working electrodes along with high biocompatibility. This developed immunosensor can quantify the concentration of MSG in the Nano-molar range with higher stability and reproducibility.

1.4 Contribution of the Thesis

This work focused on developing metal nanomaterials with hydrophobic characteristics and their deposition through the Langmuir-Blodgett (LB) film process. It is expected that a monolayer of metallic nanomaterials with enhanced conductivity could provide a better choice for effective transportation of charge and improved biomolecule adsorption capabilities compared to other methods and materials used for developing immunosensor platforms. An immunosensor based on the monoclonal anti-glutamate antibodies immobilization onto a thin film of new nanocomposites of polymer-metallic nanoparticles, conductive polymer-metal oxide nanoparticles, and conductive polymer-metallic nanoparticles through a self-assembly process was developed. New platforms developed in this thesis concurrently manage functional groups' availability for antibody immobilization with enhanced electrical conductivity. An antibodies-based immunosensor is ideal for industrial applications since it allows for detecting MSG at concentrations ranging from nanomolar to micromolar on a single platform. A hypothesis for low-concentration detection is proposed by considering the following facts: -

In-situ reduced chitosan gold nanoparticles matrix provides both higher sensitivity and extended limit of the detection range of MSG. It is expected that the impregnation of the in-situ reduced gold nanoparticles in the chitosan network provides better charge transportation and enhanced selective functionalization capability and biocompatibility. The proposed in-situ reduced gold nanoparticles may have better chemical coordination and homogeneous distribution in the chitosan network. Thus, this reduction process has an advantage over the methods that adopt the physical mixing of nanoparticles with polymeric materials. The formulation also has the advantage of stable and adhesive coatings on working electrodes to develop a platform based on monoclonal anti-glutamate antibodies immobilization. Electrochemical characteristics of Chitosan- gold nanoparticles were studied by measuring relative change in redox current compared to standard working electrodes.

The second method uses the titanium dioxide (TiO_2) nanoparticles in the PANI matrix and optimizes conditions for preparing LB films of the polyaniline-TiO_2 nanocomposite. In this study, It was adopted a unique strategy in which (i) electrochemical polymerization of aniline in the presence of TiO_2 nanoparticles was performed on ITO substrate, (ii) dissolving it in a solution of NMP and isopropanol, and (iii) lastly, LB film deposition of contamination-free subphase of PANI-TiO_2. Thus, the work achieved a higher degree of polymerization through the electrochemical process and subsequently used this polymerized material to make LB film. Electrochemical characteristics of PANI-TiO_2 were explored by measuring relative change in redox current compared to standard working electrodes.

In the third method, the chemically synthesized surfactant-free stable ultra-small gold nanoparticles (< 10 nm diameter) in the PANI matrix were used. Electrochemical polymerization of aniline monomers with these gold nanoparticles was performed on the ITO substrate. This unique method enabled the synthesis of polyaniline-GNP nanocomposite with hydrophobic nature. Further, this PANI-GNP nanocomposite was dissolved in a solution of NMP and isopropanol, and the conditions were optimized for preparing LB films of the polyaniline-GNP nanocomposite. The developed thin film shows a very high conductivity with improved capability for the adsorption of bio moles. The developed electrochemical biosensor shows stability over a wide range of pH values. The electrochemical characteristics of PANI-GNP were explored by measuring relative change in redox current compared to standard working electrodes in the electrolyte and the food sample.

The electrochemical properties of all the immunosensors mentioned above were investigated by measuring the relative change in redox current compared to standard working electrodes. To improve measurement sensitivity, monoclonal anti-glutamate antibodies were bound to the amine-functionalized spots on the working electrode using the carbodiimide coupling technique.

Since monoclonal anti-glutamate antibodies were used due to their ability to bind the uniquely designed substrate and arrange reactive groups in a manner that promotes a specific reaction transition state, they give these antibodies their selectivity. Monoclonal anti-glutamate antibodies show stereo selective properties (e.g., the monoclonal anti-glutamate antibodies used in the work reported in this thesis are specific for the L-isomer of glutamate). Anti-glutamate monoclonal antibodies are also stereo selective. The activity of monoclonal anti-glutamate antibodies can be controlled and retained by immobilizing them on biocompatible nanocomposite materials used for developing immunosensor platforms.

1.5 Structure of the Thesis

This introduction further explains what was predicted in the body of this thesis. Following is a description of the thesis's structure;

A comprehensive review of the use of various traditional methods for determining MSG is provided in Chapter 2. These approaches are divided into four categories: non-enzymatic strategies, enzymatic techniques, antibodies-based approaches, and other approaches. Both glutamate oxidase-based biosensors and glutamate dehydrogenase-based biosensors are under the category of enzyme-based methods for determining MSG.

Three unique combinations of materials are planned (i) the use of conducting material in a biocompatible polymer which has a range of functional moieties to find with antibodies, (ii) the use of semiconducting material with conducting polymer, and finally, (iii) using conducting materials with conducting polymer. These studies are described in Chapter 3 and Chapter 4. The chemical process for synthesizing nanocomposites such as chitosan-mediated reduction of GNP, in-situ reduction of dual-metallic nanoparticle conjugates, and chemical synthesis of GNP are described in chapter 3 with their detailed Physio-chemical characteristics. Chapter 4 outlines the electrochemical polymerization process used to produce nanocomposites. These processes can be categorized as the electrochemical polymerization of PANI, PANI TiO2 nanocomposite and its LB film deposition, and the electrochemical polymerization of PANI-GNP nanocomposite and its LB film deposition process. Chapter 5 discusses the electrochemical detection of monosodium glutamate using the nanocomposites GNP-chitosan, LB film of PANI-TiO2, and LB film of PANI-GNP. Finally, the conclusion and future scope of the work have been discussed in Chapter 6.