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# Use of Nanomaterials in the Detection of Food Contaminants

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#### Author's contribution

This work was carried out in collaboration between all authors. Authors SKS and SSA designed the structure of review and wrote the first draft of the manuscript. Authors SSA, JGL and NJ revised the manuscript and contributed ideas throughout the review and helped with manuscript revision. All authors read and approved the final manuscript.

**Review Article** 

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# ABSTRACT

Food safety plays an important role in public health and thus to society as a whole. Food borne illness associated with toxins, pathogens or other food contaminants poses a serious health threat all over the world. Food may become unsafe due to the presence of adulterants such as melamine, food born pathogenic bacteria, toxins such as cholera, shiga, aflatoxin amongst others. The present review is focused on the potential role of nanomaterials, which are currently being used in various biosensors, for the detection of various chemical contaminants, toxins and pathogens.

Keywords: Novel nanomaterials; nano sensors; biosensors; food safety.

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# **1. INTRODUCTION**

Nanotechnology is an area of rising interest and opens up incredible new possibilities for all industries, including food production. Food safety is one of, if not the most important aspects of public safety because food is an essential part of human life. With increasing knowledge and health awareness, consumers are now demanding food products which are free from chemical, biological, physical and radiation contamination which comes through processing, handling and distribution of most foods.

Food packaging with nano materials may enhance food safety by being used as biosensors to alert consumers about contamination of food or spoilage, tears in packaging, and even help with the controlled release of preservatives extend the shelf life of foods. Nanotechnology applications in the food industry can be advantageous in detecting bacterial contaminants on packaging or to produce stronger flavors and improve color quality, and increasing safety by increasing the barrier properties. Nanotechnology holds great promises to provide benefits not only within food products but also around food products (packaging). Thus nanotechnology introduces new chances for innovation in the food industry at immense speed [1].

Nanomaterials are materials with at least one dimension smaller than 100nm which are further classified as: i) nanofilms and coatings (<100nm in one dimension), ii) nanotubes and wire (<100nm in two dimensions) and iii) nanoparticles (< 100nm in three dimensions) [2]. Because of the incredibly small size, nanomaterials display unique physical and chemical features.

Nanomaterials are mostly used in various biosensors, nanosensors, and immunosensors which can help to detect at very sensitive levels toxic contaminants that can arise from environmental contamination during processing or handling [3,4]. The present review will focus on the mechanism of action of nanomaterials with toxic or microbial components and their potential applications as novel nanoscale sensors which could help in detecting food contaminants early and thus be an important tool for solving food safety issues.

# 2. DETECTION OF CHEMICAL CONTAMINANTS IN FOOD

Chemical contaminants are unwanted harmful substances which are intentionally or unintentionally added to foods that can come from natural sources (prime materials), environmental pollution or be formed during food processing. There are various chemical contaminants such as acrylamide, heavy metals, pesticides (such as DDT or dichlorodiphenyl trichloroethane) which may show adverse effects on health. Traditionally, high performance liquid chromatography (HPLC), liquid chromatography/mass spectrometry, gas chromatography/mass spectrometry (GC–MS), enzyme-linked immune sorbent assay (ELISA) and capillary electrophoresis have been developed for the detection of toxic these types of contaminants. These methods have high sensitivity and good accuracy, but require complicated and time-consuming steps, expensive instrumentation and specific skills from trained personnel which strongly limit their wider application. Thus new methods such as nano-sensors could be used as a more efficient tool for the detection of food contaminants. At continuation, the use of nanomaterials for the detection of the most predominant chemical contaminants will be discussed. Table1 summarizes the role of various nanomaterials in the detection of contaminants such as melamine, carbofuran with their sensitivity.

# Table 1. Use of various nanomaterials in detection of food contaminates

Nanomaterials	Food Contaminant	Electrode/ Sensor	Sensitivity	Reference
Gold nanoparticles	Melamine	Colorimetric probe	0.4 mg/L.	[8]
Au NP	Melamine	Standard colorimetric card	1-120 mg/L	[13]
Gold nanoparticles	Melamine	Surface enhanced Raman spectroscopy (SERS)	100–200 µg/L	[14]
Water-soluble CdTe quantum dots	Melamine	Fluorescence probe	0.04 mg/L	[15]
Single-wall carbon nanotube (SWNT)	Melamine	Electrochemiluminiscence	1×10 <sup>-13</sup> M	[22]
Gold nanoparticle and PB-MWCNTs-CTS	carbofuran	electrochemical immunosensor	0.1 - 1 µg/mL	[27]
Carbon nanotubes (CNTs)	Foodborne Bacterial Pathogens (Salmonella)	Electrochemical biosensor	1.6×10 <sup>4</sup> cfu/ml	[39]
Magnetic nanoparticles & TiO <sub>2</sub> nanocrystals	Salmonella	Optical nanocrystal probes	100 cfu/ml	[40])
Oligonucleotide-functionalized Au nanoparticles	Escherichia coli O157:H7	Piezoelectric biosensor	1.2×10 <sup>2</sup> cfu/ml	[43]
Glyconanoparticles	Cholera toxin	Colorimetric bioassay	54 nM (3 µg/mL)	[48]
Liposomic and poly(3,4- ethylenedioxythiophene)-coated carbon nanotubes	Cholera toxin	Electrochemical immunosensor	10 <sup>(-14)</sup> -10 <sup>(-7)</sup> g mL <sup>(-1)</sup>	[49]
Gold nanoparticle	Staphylococcal Enterotoxin B (SEB)	Chemiluminescence (ECL) immunosensor	0.01 ng/mL	[52]
Carbon nanotube	Staphylococcal Enterotoxin B (SEB)	Immunosensor	0.1 ng/mL to 100 ng/mL	[53]
AuNP-PAADs	Brevetoxins	Electrochemical immunosensor	0.03–8ng/mL	[56]
Functionalized-gold nanoparticles	Aflatoxin	Immunoelectrode	10–100 ng dL-1	[57]
Silver core and a gold shell (AgAu)	Aflatoxin B <sub>1</sub>	Immunodipstick assay	0.1 ng/mL	[58]
Antigen-modified magnetic nanoparticles (MNPs) and antibody functionalized upconversion nanoparticles (UCNPs)	Aflatoxin B1(AFB1) and ochra- toxinA(OTA)	Immunosensing probes and signal probes.	0.01 to 10ng/mL	[59]
Nanostructured zinc oxide	Mycotoxin	Indium-tin-oxide (ITO) glass plate	0.006–0.01 nM/ dm <sup>3</sup>	[61]
Single-walled carbon nanotubes (SWNTs)	Ochratoxin A (OTA)	Fluorescent aptasensor	25 nM - 200 nM	[62]
Fe <sub>3</sub> O <sub>4</sub> NPs	Campylobacter jejuni	Glassy carbon electrode	1.0 × 10 <sup>3</sup> to 1.0 × 10 <sup>7</sup> CFU/mL	[66]

#### 2.1 Melamine

Melamine (1, 3, 5-triazine-2, 4, 6-triamine) is a commercially synthesized organic compound that is produced from urea releasing cyanic acid along the way [5]. Melamine has shown to produce acute toxicity, chronic toxicity and urinary stone formation using animal models [5]. Foods that contain melamine because of adulteration have been proven to be responsible for hundreds of pet deaths [6]. In September 2008, there were a series of international media reports that showed that some infant formulas were illegally adulterated with melamine in China which led to health problems for thousands of infants. After investigation, it was shown that the inclusion of melamine to food can boost the apparent protein content, since melamine contains 66% nitrogen by mass and the most common protocols for protein detection in fact determine nitrogen content. These adulterations are very dangerous since melamine can lead to the formation of insoluble melamine cyanurate crystals in kidneys causing renal failure and could also damage the reproductive and urinary systems of animals [7]. The World Health Organization (WHO) has recommended that the tolerable daily intake for melamine be less than 0.2mg melamine per kg of body mass.

Currently, gold nanoparticles, cadmium telluride (CdTe) quantum dots and single wall carbon nanotubes combined with various analytical methods (Table 1) are being used for the rapid detection of melamine in different food matrices [8,9].

A visual method for the detection of melamine in raw milk using gold nanoparticles (GNPs) was reported by Li [8]. In this method, when a gold nanoparticle colloidal solution reacts with melamine, a change in color from red to blue is observed without the need of any equipment. A representation of the mechanism for the detection of melamine using gold nanoparticles is shown in Fig. 1. Gold nanoparticles carry negative capping agents (citrate ions) which are responsible for electrostatic repulsion against Van der Waals attraction between GNPs so that they are stabilized against aggregation in agueous solution [9]. GNPs in solution displays a red colour due to the collective oscillations of the surface electrons induced by visible light of suitable wavelength, which is highly dependent on inter particle distance [10,11]. When the interparticle distance is shortened, the solution turns blue. This happens in the presence of the amine functional groups  $(-NH_2)$  present in the melamine which easily binds with GNPs [12]. Based on this principle a reliable and sensitive kit for the rapid detection of melamine using gold nanoparticle to analyze various milk and dairy products has been developed by Zhou [13]. This kit was stable at room temperature and samples analyzed by the kit were similar to results obtained by high-performance liquid chromatography/mass spectrometry (Table 2). It is also applicable to qualitative and semiquantitative field detection, as well as naked-eye screening without the need to use any instrumentation. The first portable surface enhanced Raman spectroscopy with gold nanoparticles to selectively screen melamine adulteration in a variety of food and pharmaceutical matrices, including milk powder, infant formula, lactose, whey protein, wheat bran and wheat gluten was developed by Mecker [14].

Table 2. Melamine Detection (mg/L) in Milk Samples using gold nanoparticles (Rapid	d
Kit) and HPLC/MS (n=3, mg/L) adopted from Zhou et al. (2011) [13]	

Concentration of melamine (mg/L)	Detection using the Rapid Kit	Detection using HPLC/MS
0	0, 0, 0	0.01±0.01
12.5	15, 15, 15	12.20±0.38
25.0	30, 30, 30	23.57±2.36
50.0	60, 60, 60	44.25±2.09

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Fig. 1. Representation of the GNP colorimetric mechanism for melamine detection (Figure is adapted from Li [8] with permission)

Detection of melamine using nanoparticles could also be possible using fluorescence detection. Water-dispersable cadmium telluride (CdTe) quantum dots (QD) capped with thioglycolic acid (TGA) was synthesized by Zhang to investigate their interaction with melamine [15]. It was shown that the concentration of melamine was directly proportional to the quenching intensity of fluorescence of TGA-CdTe QD. The reason that melamine causes quenching of fluorescence emission by QD could be due to energy transfer [16], charge diversion [17] or surface absorption [18], all of which could cause a change in the surface state of the nanoparticles. Both melamine and cyanuric acid can cause quenching the fluorescence emission of TGA-CdTe QDs because cyanuric acid contains three hydroxy groups (-OH) instead of the three amine groups (-NH<sub>2</sub>) in melamine. A possible mechanism to explain the interaction between TGA-CdTe QD and melamine is represented in Fig. 2. In this experiment, hydroxy groups (-OH) and amine groups (-NH<sub>2</sub>) can form hydrogen bonds with the carboxyl groups on the TGA-CdTe QD surface [19,20], thus melamine and cyanuric acid could bind to the surface of TGA-CdTe QD forming a molecular cap through the hydrogen-bond interaction which in turn induces the quenching of QD fluorescence [21].

Similarly, single-wall carbon nanotubes (graphene) modified with glassy carbon was used for the detection of melamine using electrochemiluminiscence by Liu [22]. Using this method the sensitivity for melamine obtained was 1×10<sup>-13</sup> M. Because of costs, graphene has been used as a partial replacement for gold nanoparticles. Recently Medina (2011) reported the tuning of carrier concentrations in graphene by the molecular doping of melamine on its surface. This suggests high possibility of graphene application for detection of melamine in the near future [23].

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Fig. 2. Schematic representation of the proposed mechanism for the quenching effect of melamine on the fluorescence of TGA-CdTe QDs. Image adapted with permission from Zhang [15]

# 2.2 Ampicillin

Ampicillin, which belongs to the penam class of Beta-lactam antibiotics, has been used extensively in medicine and agriculture to treat bacterial infections and to increase animal growth. It is effective against a number of bacteria including *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Escherichia coli, Salmonella*, and *Shigella*. However, the misuse of ampicillin in animal husbandry and in many household cleaning products have led to the appearance of undesirable residues in food products which in turn can cause severe health problems, such as allergic reactions, breathing difficulties, and seizures in humans.

Songs [24] developed Aptasensor for the detection of ampicillin using a gold nanoparticle (GNPs) based dual fluorescence–colorimetric method. The selected aptamers, AMP4 (5'-CACGG-CATGGTGGGCGTCGTG-3'), AMP17 (5'-GCGGGCGGTTGTATAGCGG-3'), and AMP18 (5'-TTAGTTGGGGTTCAGTTGG-3'), were confirmed to have high sensitivity and specificity to ampicillin. In the described work solution, AMP17 ssDNA aptamers were used for adsorption onto the surface of the GNP. Because of the specific interaction between the ampicillin and the aptamer, the presence of ampicillin caused the aptamer to release the GNP and as a result, the liberated GNPs were aggregated in the presence of salt causing a colour change from red to purple. Since it was shown that the higher the concentration of ampicillin in the sample, e more free GNP were present in solution, making this method an interesting alternative for the detection of ampicillin in the food chain.

# 2.3 Carbofuran

Carbofuran is widely used pesticide to control insect and nematode pests on a variety of agricultural crops, due to their wide-range biological activity and relatively low persistence compared with organochlorine pesticides [25]. As a result of the extensive use of pesticides for agricultural and non-agricultural purposes, the environmental contamination has been directly responsible for many human health issues. Carbofuran poisoning includes nausea, vomiting, abdominal cramps, sweating, diarrhea, excessive salivation, weakness, imbalance, blurred vision, breathing difficulty, increased blood pressure, and incontinence. Death may result at high doses from respiratory system failure associated with carbofuran exposure [26].

An electrochemical immunosensor has been developed for the detection and quantification of carbofuran using GNPs, prussian Blue-multiwalled carbon nanotubes-chitosan (PB-MWCNTs-CTS) nanocomposite film and protein A (SPA) assembled using layer-by-layer (Table 1) [27]. Films where GNP and PB-MWCNTs-CTS were incorporated enhanced the electroactivity and stability of this new immunosensor. The porous three-dimensional PB-MWCNTs-CTS nanocomposite film provided many amino groups and carboxyl groups to cross-link SPA and offered a large specific surface area to immobilize SPA. The formation of a self-assembled SPA layer was employed onto the electrode surface to increase the binding capacity of the antibody [27].

# 2.4 2,4-Dinitrophenol

2,4-Dinitrophenol (DNP) was formerly used in body weight control to increase fat metabolism. DNP was used extensively in the 1930s in diet pills. By the end of 1938 it was shown to be the cause of fatal fever in United Stated forcing the stop of its use. Over dosage of DNP have been responsible for death due to extremely high raises in body temperature, this would be due to the fact that in living cells, DNP loses energy in the proton gradient in the form of heat instead of producing ATP [28]. Ko and his team [29] have developed a colorimetric nanobiosensor based on the chromogenic effect of latex microspheres hybridization with gold nanoparticles. A toxin analog, 2, 4-dinitrophenol–bovine serum albumin (DNP–BSA) was attached with GNP which allowed the hybridation of DNP with an anti-DNP antibody on latex microspheres resulting in the formation of pinkish-red color.

A model toxin, DNP–glycine was detected and quantified via a competition that occurs between the analog-conjugated-GNP and the toxin molecules for the binding pocket in the anti-DNP antibody. When the gold nanoparticles were displaced from host latex microspheres in the presence of the toxin molecules, a visible color change occurred from pinkish-red to white.

# 2.5 Dichlorodiphenyltrichloroethane

DDT chemically known as 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane was one of the most widely used organochlorine pesticides in the world when its insecticidal properties were first recognized by Muller in 1939 [30]. Because of this high efficiency, more than one million tons of this organochlorine pesticide has been used worldwide [31]. DDT is now banned in many countries due to its deleterious effects on wildlife, and human and to its persistence in the environment.

A gold nanoparticle based dipstick competitive immune assay was developed to detect organochlorine pesticide such as DDT at the nanogram level [32]. GNP with specifically defined sizes were synthesized and conjugated to anti-DDT antibodies, which served as the detecting reagent. DDA (1,1,1-trichloro-2,2-bis(chlorophenyl)acetic acid) -BSA conjugate, used as the antigen, was immobilized on nitro cellulose (membrane strips [32]. GNP conjugated anti-DDT antibodies were treated with free DDT to form an immunocomplex which then with the DDA-BSA conjugate. Depending on the concentration of free DDT in the sample the binding of GNPs conjugated anti-DDT antibodies to the immobilized DDA-BSA varied and was detected by the development of red color (due to the gold nanoparticles) and the intensity of color development was inversely proportional to the DDT concentration with maximum intensity at zero DDT concentration [32].

# **3. DETECTION OF BACTERIAL TOXINS IN FOOD**

The detection and prevention of food borne pathogenic bacteria are obviously very important in maintaining humans and other animals healthy. *Campylobacter, Salmonella, Listeria monocytogenes, Escherichia coli (E. coli)* O157:H7, *Staphylococcus aureus*, and *Bacillus cereus* are the most common pathogen bacteria which are responsible for the majority of foodborne illness outbreaks [33-35].There are many biological and immunological assays available for the detection of bacterial toxins such as sensitive ELISA-based tests, but these are not able to measure the functional activity of the toxin. Highly specific and sensitive DNA probes and PCR-based tests have been described which can detect potentially toxigenic strains of bacteria, but a positive result does not indicate that toxin gene was actually expressed [36]. Hence, nanomaterials have to great potential to be incorporated into various sensors to develop rapid, sensitive, specific method for detecting foodborne pathogenic bacteria ensuring food safety and security and to be efficient alternatives over the existing traditional methods which are laborious and time consuming. Role of various nanomaterials in detection of microbial toxin is summarized in Table1.

# 3.1 Salmonella and Escherichia coli

Salmonella are responsible for food poisoning with symptoms like diarrhea, fever, abdominal cramps, whereas E. coli can cause symptoms like bloody diarrhea, stomach cramps, nausea and vomiting [37,38]. Jain et al. [39] developed a biosensor by incorporating carbon nanotubes (CNTs) for the detection of Salmonella [39]. Salmonella monoclonal antibodies were covalently attached onto the high surface area of CNTs by using diimide activated imidation coupling then immobilized onto a glassy carbon electrode. The presence of the pathogen was detected by studying the changes in charge transfer resistance and impedance, before and after the formation of an antigen-antibody complex. This is possible because CNTs behave as molecular wires allowing electrical communication between the underlying electrode and the conjugated antigen-antibody complex. Joo [40] detected Salmonella bacteria in milk by using a antibody-conjugated magnetic nanoparticles (MNPs) and separated them from the samples by applying an external magnetic field. The MNP-Salmonella complexes were re-dispersed in a buffer solution then exposed to antibodyimmobilized TiO2-nanocrystal (TNs), which absorb UV light. After magnetically separating the MNP-Salmonella-TN complexes from the solution, the UV-Vis absorption spectrum of the unbound TN solution was obtained and since absorption was reversely proportional to the Salmonella concentration, the assay exhibited high sensitivity against low concentrations of Salmonella bacteria.

Amine-functionalized magnetic nanoparticles (AF-MNPs) were used by Huang to efficiently remove between 88.5 and 99,1% of bacterial pathogens from water, food matrixes, and urine samples [41]. The mechanism involved in this experiment was the positive charges on the surface of AF-MNPs which promoted strong electrostatic interactions with negatively charged sites on the surface of bacterial pathogens thus exhibited efficient adsorption of at least eight different species of Gram-positive and Gram-negative bacteria. The amount of AF-MNPs, pH of phosphate buffer solution, and ionic strength were crucial in mediating fast and effective interactions between AF-MNPs and bacteria. However, although very interesting, this method needs to be studied not only with pathogenic bacteria, but also beneficial bacterial to demonstrate its specificity.

Maurer [42] designed a multi-step sensor assembly for the rapid and selective detection of *Escherichia* (*E.*) *coli*. The assembly was accomplished by growing carbon nanotubes on a graphite substrate, the direct addition of gold nanoparticles on the nanotube surface, and the attachment of *E. coli* specific thiolated RNA to the bound nanoparticles. He reported that compounded nano-materials had the distinct advantage of retaining the electrical behavior property of carbon nanotubes, through the gold nanoparticles. The increased surface area through the use of nanotubes for additional analyte attachment sites, increase the methods sensitivity. These RNA coated gold nanoparticles were shown to enhance *E. coli* detection by 189% when compared to bare gold nanoparticles.

Chen et al. [43] developed a piezoelectric biosensor using oligonucleotide fused GNPs to rapidly detect the foodborne pathogen *E. coli* O157:H7 [43]. A synthesized thiolated probe specific to *E. coli* O157:H7 *eaeA* gene was immobilized on to the surface of piezoelectric biosensor. Hybridization was induced by exposing the immobilized probe to the *E. coli* O157:H7 eaeA gene fragment (104-bp) amplified by PCR, resulting in a mass change and a consequent frequency shift of the piezoelectric biosensor. A second thiolated probe, complementary to the target sequence, was conjugated to the GNPs and used as a "mass enhancer" and "sequence verifier" to amplify the frequency change of the piezoelectric biosensor. The PCR products amplified from concentrations of 120CFU/ml of *E. coli* O157:H7 were detectable by the piezoelectric biosensor. Although this method does not give instant results, it is highly specific and is lot quicker than standard plate counts that can take up to 48h for growth and cannot differentiate between regular *E. coli* and the pathogenic strains such as O157:H7.

So et al. [44] reported the use of multiple arrays of aptamer-functionalized single-walled carbon-nanotube field-effect transistors (SWNT-FETs) for the detection of *E. coli*. This method showed high sensitivity for sensing *E. coli*, which showed a conductance decrease of more than 50% after binding [44].

# 3.2 Vibrio cholera

Cholera toxin (CT) is categorized in the  $AB_5$  toxin category of family [45] which is secreted by the bacterium *Vibrio cholerae* and is the major virulence factor of cholera [46]. Cholera is an acute infection affecting the intestine, characterized by copious watery diarrhea that can lead to severe dehydration and ultimately death if treatment is not given promptly [47] and is normally caused by consuming contaminated food or water. Schofield [48] developed glyconanoparticles for the detection of Cholera toxin. The colorimetric bioassay is depending on a specifically synthesized lactose derivative which is self-assembled onto GNPs. In solution the lactose-stabilized nanoparticles were red in color due to the intense surface plasmon absorption band centered at 524nm and when the Cholera toxin bound to the lactose derivative, this resulted in aggregation of the nanoparticles and the formation of a deep purple color. The selectivity of the bioassay stems from the thiolated lactose derivative that mimics the GM1 gangliosides to the receptor to which cholera toxin binds in the small intestine. The stability of the lactose-stabilized nanoparticles was established by freezedrying and then resuspending the particles in water and subsequently measuring the toxin in biologically relevant electrolyte solutions. This colorimetric bioassay provides a new tool for the direct measurement of cholera toxin.

A sensitive method for the detection of cholera toxin using an electrochemical immunosensor with liposomic and poly (3,4-ethylenedioxythiophene)-coated carbon nanotubes was demonstrated by Viswanathan [49]. The toxin was detected by a "sandwich-type" assay on the electronic transducers, where the toxin is first bound to the anti-toxin antibody and then to the GM<sub>1</sub>-functionalized liposome. Adsorptive square-wave stripping voltammeter was utilized to measure the released electroactive marker. This sandwich assay provides the amplification route for the detection of the toxin present in ultratrace levels (detection limit of this immunosensor was  $10^{(-16)}$ g of cholera).

# 3.3 Staphylococcal Enterotoxin B

Staphylococcal enterotoxins (SEs) form a group of twenty-one heat stable toxins that can cause foodborne diseases, even at exposure levels as low as 20–100 ng per person, resulting from consumption of contaminated foods [50-51]. Yang et al. (2009) developed a chemiluminescence mmunosensor using GNPs which enhances detection of Staphylococcal Enterotoxin B (SEB) in food [52]. Immobilization of anti-SEB primary antibodies onto a gold nanoparticle surface through physical adsorption and then the antibody–gold nanoparticle mixture was immobilized onto a polycarbonate surface. The toxins were detected by a "sandwich-type" ELISA assay on the polycarbonate surface with a secondary antibody and ECL detection. The signal from ECL was read using a point-of-care detector based on a cooled charge-coupled device (CCD) sensor. The limit of detection was found to be ~0.01ng/mL, which is 10 times more sensitive than traditional ELISA.

Similarly anti-SEB antibodies were immobilized onto a CNT surface through electrostatic adsorption and then the antibody–nanotube mixture was bound onto a polycarbonate film for detection of Staphylococcal Enterotoxin B (SEB) in food [53].

# 3.4 Brevetoxins

Brevetoxins (BTXs) are cyclic polyether compounds produced naturally by a species of dinoflagellate known as *Karenia brevis* and are potent marine neurotoxins that bind to voltage-gated sodium channels in nerve cells, leading to disruption of normal neurological processes and causing the illness clinically described as neurological shellfish poisoning [54]. Wang [55] confirmed BTX-B2 and S-desoxy-BTX-B2 as the most abundant of B-type brevetoxin metabolites in the Eastern oyster. A sensitive electrochemical immunosensor was developed by means of immobilizing BTX-2–bovine serum albumin conjugate (BTX-2–BSA) on the gold nanoparticles-decorated amine-terminated poly(amidoamine) dendrimers (GNP–PAADs [56]. The determination of BTX-2 was performed using a competitive-type immunoassay format using horseradish peroxidase-labeled anti-BTX antibodies as trace in the system. A low detection limit (LOD) of 0.01ng/mL and a wide dynamic working linear range of 0.03-8ng/mL BTX-2 using GNP-PAADs as matrices were obtained, Importantly, this method provided a biocompatible immobilization and a promising immunosensing platform for analytes with small molecules in the analysis and detection of food safety.

#### 4. DETECTION OF FUNGAL TOXINS IN FOOD

Aflatoxin B<sub>1</sub> (AF B<sub>1</sub>) is the most common mycotoxin produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus* that can grow on food crops during their production and storage. It exhibits carcinogenic, teratogenic, mutagenic and immunosuppressive properties and has been classified as a human carcinogen by the International Agency for Research on Cancer. Aflatoxin B1 antibody (aAFB<sub>1</sub>) covalently attached to cysteamine functionalized-gold nanoparticles (C-GNP) was immobilized onto 4-mercaptobenzoic acid (MBA) based self-assembled monolayer (SAM) on gold electrode (MBA/Au) [57] as illustrated in Fig. 3. This immunosensor based on antibody attached gold-nanoparticles has been fabricated for the detection of aflatoxin B1 in the range of 10–100ngdL<sup>-1</sup>and has a response time of 60 s making it a very fast and effective detection method.

Liao and Li [58] developed a lateral flow strip for detection of aflatoxin  $B_1$  in food by using the respective monoclonal antibody immobilized on nanoparticles with a silver core and a gold shell (AgAu) as detection reagent. The membrane-based immune-dipsticks consisted of a test line containing AF  $B_1$  conjugated to bovine serum albumin, and a control line with goat anti-mouse IgG. One to two colored lines are formed on the membrane by using red AgAu nano-particles coated with anti-AF  $B_1$  as signaling reagents.

Wu et al. [59] developed a sensitive and rapid, competitive fluorescence immuno-assay for the simultaneous detection AF  $B_1$  and ochratoxin A (OTA, another frequent mycotoxin produced by *Aspergillus*) in foodstuffs using antigen-modified magnetic nanoparticles as immuno sensing probes, and antibody functionalized rare-earth-doped NaYF4 upconversion nanoparticles as multicolor signal probes. The luminescent intensity was highest in the absence of mycotoxins, and the fluorescent signals of the nanocomposites gradually decreased with increasing the concentrations of AF  $B_1$  or<OTA. The use of magnetic nanoparticles is advantageous for rapid separation and purification of the immune complex, thus reducing the overall assay time.

Nanostructured ZnO has biocompatibility which facilitates immobilization of an enzyme and protein via electrostatic interactions. Also, the positively charged ZnO nanoparticles not only provides a friendly microenvironment for immobilizing negatively charged rabbit antibodies thatretains its bioactivity, but also accelerate electron transfer communication between protein and the electrode to a large extent [60]. Nanostructured zinc oxide (Nano-ZnO) film has been deposited onto indium–tin–oxide (ITO) glass plate for co-immobilization of rabbit-immunoglubin antibodies (r-IgGs) and bovine serum albumin (BSA) for OTA detection. In this layout, positively charged Nano-ZnO binds to the carboxyl groups of r-IgG via electrostatic interactions and free amino terminal sites of r-IgG preferably bind with the carboxylic group of OTA molecules [61].

Guo et al. [62] constructed a sensitive and selective fluorescent aptasensor for OTA detection, utilizing single-walled carbon nanotubes (SWNTs) as quencher. They obtained the detection limit of 24.1nM with a linear detection range from 25nM to 200nM. This technique responded specifically to OTA without interference from other analogues (N-acetyl-L-phenylalanine, warfarin and ochratoxin B). It was verified for real sample application by testing 1% beer containing buffer solution spiked with a series of concentration of OTA proving its effectiveness in a real food sample [62].





Fig. 3. Fabrication of BSA/aAFB<sub>1</sub>-C-AuNP/MBA/Au immunoelectrode (adopted from Sharma [57] with permission)

# 5. DETECTION OF PORK ADULTERATION INTO READY TO MADE HALAL FOODS

Halal foods are those that are allowed under Islamic dietary guidelines and according to their customs, Muslim's cannot eat amongst other foods, pork or pork by-products In the last years there has been an increasing demand for ready-made Halal foods, such as burgers, pizzas, hot dogs, sandwiches, soups, cookies, candies, and creams [63]. A number of countries, such as Malaysia, Indonesia, Thailand, China, India, Australia, New Zealand, Brazil, Turkey, and Singapore, are trying to capture the huge opportunities of the global Halal food markets [55]. To survive in the highly competitive marker and to realize excessive profit, dishonest labeling of Halal foods is frequently occurring such as the inclusion of pork by-products in other meats [64].

Pork adulteration in beef and chicken meatball was detected by Ali [65] using GNPs as colorimetric sensors. GNPs change color from pinkish-red to gray purple, and their absorption peak at 525nm was red-shifted by 30–50nm in 3mM phosphate buffer saline. Adsorption of single-stranded DNA protects the particles against salt-induced aggregation. Mixing and annealing of a 25-nucleotide single-stranded DNA probe with denatured DNA of different types of meats differentiated well between perfectly matched and mismatch hybridization at a critical annealing temperature. When pork containing vials were presented in pure and mixed forms; grey color indicated aggregation and showed red-shift of the absorption peak and significantly increased absorbance in 550–800nm regimes. In case of samples that did not contain pork DNA, the probes became available due to mismatches and interacted with GNPs to protect them from salt-induced aggregation.

# 6. CONCLUSION

Nanomaterials incorporated into the various biosensors and nanosensors enable the use of biological components to react or bind with a target molecule and transduce this event into detectable signals to help in rapid detection of food contaminant. They can play a vital role in assuring food safety and help to take quicker preventive actions when required. Thus, simple visual tests can be created and color changes can easily detected by even unskilled users making for easier testing of serious food contaminants. From the above literature it is clear that gold nanoparticles and carbon nanotubes can play a very helpful role in detection of adulterants, toxins and various residues. Further research work is required to explore such nanomaterials and new ones which could be used to improve food safety.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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