1. ABSTRACT

New methods to identify trace amount of food components and/or contaminants (infectious pathogens and chemicals) rapidly, accurately, and with high sensitivity are in constant demand to prevent foodborne illnesses. Multipurpose biofunctionalized engineered nanomaterials are very promising for the detection of food components and contaminants. The unique optical and magnetic properties of the nanoscale materials are very useful in the analysis of food. The objectives of this review paper are to discuss the development of applications of nanoscale structures related to food industries and to provide an overview of available methods of detecting food components and contaminants with particular emphasis on the use of nanoparticles.

2. INTRODUCTION

The chemical composition and nature of food components affect all aspects of food quality. The major food components supply the human body with the necessary building materials and sources of energy, as well as elements and compounds that are indispensable for metabolism (1). The main components include water, carbohydrates, proteins, lipids, vitamins, and minerals as well as other compounds present in minor quantities in foods such as nonprotein nitrogenous compounds, colorants, food flavor, and additives, which have a significant impact on food quality (2). Food components determine their nutritional value, their sensory attractiveness, and development of desirable or deteriorative changes due to interactions with other
constituents and to food processing, and susceptibility or resistance to spoilage during storage. Because of the undesirable effects of food components and additives before and after food processing, there is a need for new, sensitive, and rapid detection methods.

Global changes such as increase in population, international trade in food as well as animal feed, newly emerging pathogens, and various food-processing operations are the major factors that influence the incidence and profiles of foodborne illnesses (3). Furthermore, inadvertent contamination of food is a huge threat in the food industry.

Food contaminants may be biological or chemical in nature. Biological contaminants include microbial pathogens (i.e., disease-causing bacteria, viruses, parasites, fungi), biological toxins in soil (such as mycotoxins, bacterial toxins like staphylococcal enterotoxin B, SEB), as well as marine and freshwater toxins (4-6). Chemical contaminants include pesticides (i.e., herbicides, insecticides, fungicides) (7), environmental chemicals (e.g. polychlorinated biphenyls (PCBs) (8), polycyclic aromatic hydrocarbons (PAH) and dioxins (9), and heavy metals (e.g., lead and mercury), antibiotics (10) veterinary drugs as well as food additives (i.e., organic dyes, sulfites, and food processing contaminants (e.g., acrylamide and melamine) (11-13).

Microbial food safety has emerged as a global concern because of its ultimate effect on the health of the consumer that eventually leads to financial losses in the food industry resulting from product recalls and trade barriers. Escherichia coli (E. coli), Listeria, Salmonella, Staphylococcus, and Campylobacter are some of the examples of the most frequent microbial disease causing agents (14). The detection of pathogens by conventional methods are based on cell culture which give reliable results, however, they are time-consuming because of long incubation periods (24–48 h) (15). Recently, several new techniques have been developed to detect pathogenic bacteria. These involve polymerase chain reaction (PCR) (16), immunoassay (17), optical assay (18), ATP-based bioluminescence (19), DNA-based assay (16), Enzyme-linked immunosorbent assay (ELISA) electrochemiluminescence (20), etc. Some of these techniques are greatly restricted by the long assay time, preparation step that includes initial enrichment of the desired pathogen, maintenance of strict sterile environment, lower sensitivity, the reduced specificity, the size and price of the equipment used, complicated procedures, essential pre-requisite for skilled manpower, sometimes costly preparation, and wide acceptance (21,22). Although DNA- and protein-based detection methods are quicker, these methods still require at least several hours to perform. In order to detect a few bacterial cells in a food sample, a culture enrichment step ranging from a few hours to overnight is typically required. In the food industry, such a long waiting time for the results can be costly, destructive to food, and inconvenient. The presence of foodborne pathogens, either alive or dead, can be detected by simply determining if a specific bacterial gene of interest is present. PCR is much less time-consuming than culture-based methods, and the technique is conducive to automation and high throughput. Regular PCR methods are able to detect the presence of single pathogen, whereas a multiplex PCR allows the simultaneous detection of several microorganisms of interest or multiple genes of single microorganism (23).

Natural toxins found in the environment come from organisms that thrive in the natural environment. For example, mycotoxins are naturally produced by fungal species that grow on agricultural products, such as nuts, fruits and grains, among others, both in the field, after harvest, and during storage (4). Presence of mycotoxins in beverages and foods is due to direct contamination of plants or their products or by contamination from animal feeds. The level of mycotoxins in food and food products is important for consumer protection. There are many methods that are employed to detect mycotoxins in complex food matrices, but ELISA and chromatographic methods are the most widely used (24). Most methods for the determination of a mycotoxin must rely on the correct extraction and clean up methods except ELISA which does not require cleanup and, therefore recently became very popular due to relatively low cost and ease of application (25,26). To date, new methods to achieve higher sensitivity and address other challenges that are posed by these toxins are being developed (24).

Separation techniques coupled with different detectors such as gas chromatography–mass spectrometer (GC–MS), liquid chromatography–MS, GC-flow-injection detection (GC–FID) GC-electrical chemiluminescence detection (GC–ECD), high-performance liquid chromatography–ultra visible (HPLC–UV), HPLC-flow-injection chemiluminescence (HPLC–FICL), capillary electrophoresis–chemiluminescence detection (CE–CL) immunosensors, and CL–ELISA have been used for the analysis of most of the chemical contaminants (27–33). Nevertheless, all these methods for detection of food components and contaminants have serious limitations when applied to complex food matrices. These limitations involve tedious purification steps, long analysis time, and the need for an expert that causes the process to be expensive (34–37). Thus, there has been a demand for good analytical protocols based on efficient analytical processes – sensitive, selective, rapid, inexpensive, reduced organic solvent consumption, and suitable for multi-sample screening as dictated by legislation, health authorities, and companies operating in the food market.

The field of nano science and technology is an interdisciplinary research area that has been increasing tremendously in the last decade. Nanotechnology is slowly moving out from the experimental into various practical applications and is penetrating the agriculture and the food processing industry (38). There has been considerable interest in the application of nanostructured materials for the development of sensors for food analysis or for determination of food components and contaminants. Researchers have focused on the development of sensors for the rapid detection of pathogens and other food
contaminants in food by taking advantage of the novel and unique properties of nanomaterials (Figure 1).

Nanostructured materials refer to materials or components that are made up of nanocrystalline or quasi-nanocrystalline grains or particles. These types of materials usually have much greater mechanical (static and dynamic) properties, high tenacity, as well as magnetic and degradation resistant properties (39). Nanostructured materials represent the application of nanotechnologies in the field of materials science and engineering that show promise of great impact on several areas such as biomedicine, electronics, transportation, environment, and construction. For light metal alloys (e.g., aluminum, magnesium, and titanium) it is possible to design nanocrystals with high specific properties that are very important for bioassay applications (40).

Nanomaterials are produced by using a variety of bulk materials with either organic or inorganic components (polymeric, semiconductor, and metallic) (41). Metallic nanomaterials may be composed of various metals, metal oxides, and magnetic materials (42). The composition of these materials determines their compatibility and suitability for the relevant applications. Nanomaterials can be designed in different shapes (sphere, rod, and cube shaped) (43-47), tubes (48,49), wires (50-52), films (53-54) or composites (55) of varying sizes, ranging from a few to several hundred nanometers. Among them, nanoparticles (NPs) possess several distinctive physical and chemical attributes that make them promising synthetic scaffold for the creation of novel chemical and biological detection systems (56). Nanoparticles are smaller than 100 nanometers, contain 20–15,000 of atoms, and exist in a realm that straddles the quantum and Newtonian scales (41). In addition to the large surface-to-volume ratio that favors miniaturization, NPs show unique optical, electronic, and magnetic properties depending on their core materials. Furthermore, these properties of the NPs depend on their size and shape, and vary with their surrounding chemical environment.

NP technology based on fluorescent NPs (e.g., quantum dots (QDs), magnetic (e.g., iron oxide) and metallic NPs (e.g., gold (Au), silver (Ag) NPs) (57-59) have been successfully used to image, track, and detect various infectious microorganisms (48,58,60, 61). These have been used in bioanalysis (62), immobilization of proteins and enzymes, bioseparation, immunoassays, drug delivery, and biosensors (63-68). NP can be also conjugated with different biomolecules such as peptides and proteins (52,69), antibodies (70-71), nucleic acids (72), lipids (73), carbohydrates (74), and antibiotics (75).

There is a general need to develop simple, rapid, and inexpensive detection assays and biosensors for recognition and monitoring of food components and contaminants. Determination of food composition and their beneficial or dangerous effects on human health, the presence or absence of harmful contaminants or residues, and the status of food from the farm and from the processing plants are extremely important tasks for modern analytical chemistry. To accomplish these responsibilities new technologies are applied and adopted in food analytical methods. In recent years, there has been a great progress in nanotechnology and fabrication of specifically designed nanostructures for food analysis. The feasibility of using a variety of inorganic nanomaterials including gold, silver, and zinc oxide was evaluated for food safety control (48,70,76). The combination of nanomaterials with novel detection methods and suitable techniques can lead to sensors that are useful for precision agriculture, natural resource management, early detection of pathogens and other contaminants in food products, efficient delivery systems for agrochemicals like fertilizers and pesticides, improved systems integration for food processing, packaging and other areas such as monitoring agricultural and food system security (29, 77-79).
In this review, some of the main improvement in this field over the past few years is presented and discussed. The aim is to focus on successful applications, issues, approaches, and challenges of developing nanostructures with emphasis on NPs for detection of food components and contaminants. The excellent performance of NPs for detection of food components and contaminants are described.

3. DETECTION OF FOOD COMPONENTS AND CONTAMINANTS USING NANO-SIZED STRUCTURES

3.1. Electrochemical techniques combined with nanostructures

Electrochemistry as a sensor technology provides a wide variety of possible configurations due in part to the multitude of different electrochemical techniques available, permuted with the different detection items available (e.g., enzymes, carbon nanotubes (CNTs), and NPs). Electrochemistry has demonstrated to be enormously successful in many other branches of industry (e.g., food analysis, pharmaceuticals), environmental and medical monitoring (80). Compared with MS and chromatographic techniques (i.e., GC or, HPLC), electrochemical techniques are simpler in their setup and their electronic equipment requirements; are easier to maintain and calibrate; the signals are obtained directly in situ; and, are able to provide rapid response time. However, the precision and detection limits that standard analytical methods provide cannot be matched by electrochemical techniques at this point.

Nanostructures can be used mainly for two different purposes in electrochemical techniques. First, NPs are used to increase the surface area of the electrode. This can be done by using a layer of nanomaterials that provides a suitable surface chemistry for biomolecule immobilization or for electrode modification (81-83). Multilayers of conductive NPs modified electrode could give a porous film with high specific surface area (84-85). Second, NPs and/or nanostructures are used to enhance or amplify the electrochemical signal. The catalytic properties of metal and semiconductor NPs could lower the over potential and decrease the potential for the electrochemical responses. NPs could enhance the electron transfer between the analyte and the electrode, thus sensing systems could be fabricated without electron transfer mediators (86-88). The NPs could also be used as labels in stripping voltammetry (81-82, 89). In other words, electrochemistry in the presence of NPs improves the charge transfer from an electrode to another phase (e.g., the molecule of interest or supporting electrolyte) (90-91). In addition, the chemical reaction at the electrode surface as well as the process of charge transport can be modulated and can be used as the basis of detection (92-97).

3.1.1. Amperometric techniques

Amperometry is the most commonly used electrochemical technique for biosensor development (71, 80, 90, 98). The amount of current is monitored while controlling the potential. Enzymatic reactions, which produce electroactive products such as hydrogen peroxide (H$_2$O$_2$) or aminophenyl phosphate, are among the electroactive species for amperometric measurements (90, 99-101).

Enzyme electrode is one of the examples of application of nanoscience in food processing. Glucose, dextrose, sucrose, lactose, alcohol, and some organic and inorganic acids can be listed as examples of target food components that are measured with enzyme electrodes (102-103). It is also possible to determine some contaminants such as microorganism, pesticide, food additives, and toxins using amperometric enzyme electrode. For example, Tang (90) developed an amperometric glucose biosensor based on adsorption of glucose oxidase (GOD) at the platinum (Pt) NP-modified CNT electrode. CNT/graphite electrode has better electrocatalytic activity for H$_2$O$_2$ than does the graphite electrode. Moreover, electrodeposition of Pt-NP on the surface of CNTs resulted in an increase in the current response, which was nearly four times larger than the current response of the CNT/graphite electrode. These resulted in a short response time (within 5 s) with a large dynamic range (0.1 – 13.5 mM). The group of Ren (91) investigated immobilization of glucose oxidase in silver (Ag) solution. The Ag solution increased the current measured on the enzyme electrode from 0.531 to 31.17 $\mu$A in a solution of 10 mM $\beta$-D-glucose at a decreased response time from 60 to 20 seconds. In a similar work, glucose oxidase was immobilized in silver-gold (Ag-Au) solution and then a Pt electrode was coated with the enzyme immobilized Ag-Au solution (104). The current increased from 0.32 to 19 $\mu$A cm$^{-2}$ in 10 mM $\beta$-D-glucose.

Some studies showed that NP decorated titanium nanotube array showed remarkably improved catalytic activities during the oxidation of H$_2$O$_2$. Kang (105) fabricated a Au-Pt NP-decorated titania nanotubular electrode for the detection of glucose with a detection limit of 0.1 mM at a response time of 3 s. Similarly, Pang (106) used only Pt NP-decorated CNTs and reported a detection limit of 5.7 $\mu$M with a response time of less than 3s in the range of 0.006 mM to 1.5 mM of glucose. Zhu (107) fabricated CNT/Pt NP modified carbon electrode with improved electrode properties compared with normal glucose oxidase Pt electrode. The detection limit improved from 6.7 mM to 0.83 mM; the working potential was lowered from 0.65V to 0.45V and the response time was decreased from 30s to 5s. These developments exhibited improved detection limit, faster response time, and decreased over potential effect of NPs on amperometric enzyme electrodes.

A mediatorless biosensor was developed based on the use of AuNPs. AuNPs were immobilized on glucose oxidase electrode to promote direct electron transfer whereby the NPs assumed the role of a common redox mediator (87). A new type of amperometric glucose biosensor based on silicon dioxide coated magnetic NP decorated multiwalled CNT on a glassy carbon electrode (GCE) had also been developed by Baby and Ramaprabhu (108). The sensor exhibited a linear response from 1 $\mu$M to
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30 mM with an improved detection limit of 800 nM. In another study, hemoglobin was immobilized on one-dimensional AuNPs to construct a novel amperometric biosensor for H₂O₂ and nitrite. The immobilized hemoglobin displayed a good response to the reduction of both H₂O₂ and nitrite with a detection limit of 0.24 and 0.65 nM, respectively (109).

A sensitive immuno sensor for human IgG using AuNPs/CNT hybrid platforms with horseradish peroxidase functionalized AuNP label was developed by the group of Cai (81). A linear response between 0.125 and 80 ng mL⁻¹ with a detection limit of 40 pg mL⁻¹ was recorded. The method could be easily extended to other protein detection schemes as well as in DNA analysis of food such as pathogens and genetically modified foods.

A pesticide sensitivity test based on acetylcholine esterase immobilized on AuNP/sol-gel-derived silicate network was investigated (111). The detection limit was 0.6 ng mL⁻¹ with 10% inhibition. When the enzyme electrode was not in use, it was stored at 4 °C. The sensor retained 90% of its current response even after 30 days of storage. The AuNPs exhibited a superior conductivity with amplified sensitivity and improved response of the biosensor.

In summary, amperometric enzyme electrodes are commonly used in food processing. Among these, Pt NP, AuNPs, and silicon dioxide coated magnetic NP are used for development of enzyme electrodes to determine food components and contaminants (microorganism, pesticide, food additives, and toxins) with a short response time and increased response without no sample pretreatment.

3.1.2. Voltammetric techniques

Stripping voltammetry is a pre-concentration technique, which can be used for trace analysis of food components and contaminants. The three most commonly used techniques in this category of electrochemical methods are anodic stripping voltammetry (ASV), cathodic stripping voltammetry (CSV), and adsorptive stripping voltammetry (AdSV). Even though these three techniques have their own unique features, all use two steps. In the first step (pre-concentration step), the analyte species from the sample solution is concentrated onto or into the working electrode. During this step, NPs (Au, Ag, and QDs) can be used as labels for stripping voltammetry analysis. Pre-concentration step is performed through the dissolution of the NP tag with acid treatment. The pre-concentrated analyte is stripped into the solution (112). During the second step, the pre-concentrated analyte is stripped off of the electrode through the application of a potential window. Wang’s laboratory (113) used AuNP tags which were prepared based on the precipitation of Ag on AuNPs for DNA hybridization assay; this was used in a potentiometric stripping assay for the detection of the dissolved Ag. On the other hand, Cai (81) also used AgNPs as the oligonucleotide tag to detect the DNA hybridization in their study. They performed the hybridization assay with the AgNP labeled probes and then determined the solubilized Ag⁺ ions by anodic stripping voltammetry with a carbon ultramicroelectrode. The detection limit was 1.0 x 10⁻⁶ mol L⁻¹. In another study, Wang (83) reported simultaneous electrochemical measurements of multiple DNA targets with the use of different inorganic-colloid (QDs) nanocrystal tracers. Three encoding NPs (zinc sulfide, cadmium sulfide, and lead sulfide) were used to differentiate the signals of three DNA targets in connection with stripping-voltammetric measurements. In addition to these, encoded redox beads and encoded redox rods have also been designed for simultaneous electrochemical measurements (114). Further developments in these techniques could make these available for the detection of the genetically modified foods.

The main advantages of voltammetric techniques are the sensitive response, simplicity, and low-cost of equipments. In addition, the NP modified electrodes are reusable by polishing the surface and the use of small volume samples for analysis that minimizes the effects of contamination.

3.1.3. Impedance techniques

Generally, electrochemical impedance is determined by applying an AC potential to an electrochemical cell that results in a change in the current flowing through the cell that is measured and reported. Using this method, protein/protein interaction or DNA hybridization could be monitored through the detection of either the shift in the impedance or the change in the capacitance or admittance at the bulk of the electrode interface (115).

An impedance biosensor based on interdigitated array of microelectrode was coupled with magnetic NP-antibody conjugates for the detection of E. coli O157:H7 (70). The limit of detection (LOD) was determined as 7.4x10⁶ colony forming unit (cfu) mL⁻¹ with a total detection time of 35 min. In another study, Zhang (116) used FAuNP covalently bound to the antibody for probing apolipoprotein A-I. Linear response to apolipoprotein A-I antigens was established at 0.1-10 ng mL⁻¹. The detection limit was 50 pg mL⁻¹ (corresponding to 1.8 pmol L⁻¹), which was two orders of magnitude lower than that of traditional methods. Xu et al. (117) described a novel, sensitive DNA hybridization detection protocol based on DNA-QDs nanoconjugates coupled with electrochemical impedance spectroscopy (EIS). In their study, the aminolinked single stranded-DNA (ss-DNA) probe was covalently immobilized onto a self-assembled mercaptoacetic acid monolayer modified Au electrode. After hybridization with the target ssDNA-QD nanoconjugates, EIS was used to detect the change in the interfacial electron-transfer resistance (R-et) of the redox marker, [Fe(CN)(6)](4-/3-), from solution to the transducer surface (117). QD labels on the target DNA improved the sensitivity by two orders of magnitude when compared with non-QD tagged DNA sequences (116).

Many strategies based on DNA hybridization assay using AuNPs have been developed. Most of them rely on capturing the NP on the hybridized target in a sandwich format. Improved EIS system in the presence of
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NPs is more sensitive but takes longer than some existing methods.

3.1.4. Photonic electrochemistry: Electrochemiluminescence and photocurrent

Photonic electrochemistry involves the intimate interaction of light with electrochemical processes. Illumination of the electrode-electrolyte interface produces charge-transfer events (photocurrent) as well as electrochemical reactions that produce light as a product (electrogenerated chemiluminescence) (112).

Electrogenenerated chemiluminescence (also called electrochemiluminescence (ECL)) involves the generation of species at electrode surfaces that undergo electron-transfer reactions to form excited states that emit light (118). The ECL technique has become a very powerful analytical tool. It has is widely used in many areas such as in immunoassay for food, water testing as well as for biowarfare agent detection (119). Some key studies on ECL biosensors include detection of NADH and ethanol (93) with a detection limit of 12 nM with a linear range of 5 µM to 5.2 mM, detection of perfect-matched target ss-DNA (94) from 2.4 x 10^{-14} to 1.7 x 10^{-12} M with a detection limit of 9.0 x 10^{-15} M, low-density lipoprotein (95), CdSe IgG at 0.002-500 ng L^{-1} with a detection limit of 0.6 pg ml^{-1}, glucose at 1 µM to 5 mM with a detection limit of 0.2 µM (96), lysozyme based on aptamer biosensor coupled with AuNPs at 1x10^{-13} and 1x10^{-8} M with a detection limit of 1x10^{-13} M (92).

It is well known that the photoexcitation of semiconductors lead to the formation of photocurrent (120). When the semiconductor is induced by a light source, an electron-hole pair is generated in the valence- and conduction-band levels. Transfer of the conduction band electrons to the electrode follows this process, or the filling of the valence holes with electrons supplied by the electrode, and the release of the photocurrent (121). Recently, it has been reported that QDs can be used as photoelectroactive species for the photoelectrochemical sensing of acetylcholine esterase (AChE) inhibitors. The QD/AChE hybrid system was immobilized on the electrode. This system makes use of the biocatalyzed hydrolysis of acetylthiocholine to acetic acid and thiocholine. Thiocochline acts as an electron donor for the generation of a photocurrent in the system that is blocked in the presence of AChE inhibitors. The system generated photocurrent for the sensing of the AChE inhibitors and gives more sensitive results (121). Similarly, the photocurrent from QDs immobilized on Au electrodes was enhanced in the presence of cytochrome c (122). In this study, a photoelectrochemical signal that was sensitive to the presence of superoxide radicals was developed using CdSe/ZnS QDs that were immobilized on gold electrodes using a dithiol compound. Different surface modifications on the quantum dots using mercaptopropionic acid, mercaptosuccinic acid and mercaptopyridine led to linear response between 0.5 mM and 4.5 mM cytochrome c solutions. In another study, quantitative detection of DNA was performed using DNA-cross linked CdS NP arrays (123). Thiolated oligonucleotide-functionalized Au electrode and QDs were used in the study. A photocurrent generation was observed due to the formation of DNA cross-linked arrays in the presence of the target DNA. The system was improved in sensitivity. Bas and Boyaci (124) developed a bioaffinity sensor that detected the biotin conjugated CdSe/ZnS (core/shell) QDs (as the photoelectrochemical label) on avidin immobilized ITO electrodes quantitatively. A linear relationship between the photocurrent and biotin-conjugated QDs concentration was obtained. In this study, QDs were successfully used as labels for the photoelectrochemical biosensor.

In summary, applications of QDs as photoelectrochemical label for different biosensors resulted in higher sensitivity with a short response time for the detection of toxic compounds. This NP-associated system may be further developed for monitoring other contaminants as well as genetically modified foods.

3.2. Microgravimetric techniques combined with nanostructures

The Quartz Crystal Microbalance (QCM) is a mass sensor that measures the frequency of oscillations in the quartz crystal. Significant research on the use of QCM biosensor for the detection of various analytes like toxins and microorganisms in food has been reported (125).

Generally, AuNPs are used as signal amplifier in many microgravimetric studies. For example, the group of Su (126) developed an ultrasensitive microgravimetric biosensor using Au conjugate as a biocatalytic probe in which primary Au NP-amplified sandwich immunoassay was followed by a Ag staining reaction. The sensor elements were 10 MHz AT-cut quartz crystals coated with Ag electrodes. An immunochemical molecular recognition event between anti-human IgG (a-h IgG) and human IgG (h IgG) was chosen to illustrate the sandwich procedure and, sequentially two orders of magnitude improvement for IgG quantification was achieved. Shen et al. (127) developed an AuNP network-type thin film to avoid nonspecific interactions and to provide maximum binding to the specific target. A functional mannose self-assembled monolayer in combination with lectin Con A was used as molecular recognition elements for the detection of E. coli W1485 using QCM as transducer. The results showed a significant improvement on the sensitivity and specificity of the carbohydrate QCM biosensor with a detection limit of a few hundred bacterial cells. The linear range was from 7.5 x 10^{2} to 7.5 x 10^{7} cells mL^{-1}, which was four decades wider than the mannose QCM sensor. In another study, Xia (128) developed a nucleic acid biosensor to detect Staphylococcus epidermidis (S. epidermidis) via Au NP signal amplification. For this purpose, thiolated probes that were specific to S. epidermidis 16S rRNA gene were immobilized on the surface of QCM nucleic acid biosensor. Hybridization of the immobilized probes with the PCR amplified fragments of S. epidermidis resulted in a frequency shift due to the mass change on the sensor surface. Moreover, the frequency shift was enhanced by the conjugation of the streptavidin coated AuNPs to the PCR amplified fragments. The results showed that the lowest detection limit achieved was 1.3x10^{1} cfu mL^{-1} (128). In a
similar study, a second thiolated probe complementary to the target sequence was conjugated to AuNPs and used as ‘mass enhancer’ to amplify the frequency change of the piezoelectric biosensor (129). They reported that the PCR products corresponding to $1.2 \times 10^7$ cfu mL$^{-1}$ of *E. coli* O157:H7 cells were detected. Wang et al. (130) have presented a QCM biosensor for real-time detection of *E. coli* O157:H7 DNA using AuNPs amplification. They observed that the outer avidin-coated AuNPs was combined with the target DNA to increase the mass. The target DNA corresponding to $2.0 \times 10^9$ cfu mL$^{-1}$ *E. coli* O157:H7 cells was detectable using this system. In a similar study, streptavidin conjugated Fe$_3$O$_4$ NPs were used as mass enhancers (131). It was observed that the sensor was able to detect synthesized oligonucleotides and *E. coli* O157:H7 cells as low as $10^{-12} \text{ M}$ and $2.67 \times 10^2$ cfu mL$^{-1}$, respectively. Liu et al. (82) modified a QCM sensor surface with AuNPs to increase the available surface capture area, then enhanced the hybridization signal with gold nanoparticles derivatized with thioltated complimentary DNA. The DNA detection limit of $10^{-16} \text{ M}$ was obtained while Willner (132) reported $3 \times 10^{-16} \text{ M}$ for single-base mismatch by applying amplification by means of catalytic precipitation using alkaline phosphatase. In another study, Zhou et al. (133) fabricated a sandwich ssDNA probes/complimentary ssDNA probes modified with AuNPs/ssDNA of *Listeria monocytogenes* (*L. monocytogenes*). They reported a detection limit as low as 10 cfu mL$^{-1}$.

In summary, to enhance detection sensitivity, AuNP was employed in QCM by either hybridizing NP-oligonucleotide conjugates to complimentary surface-immobilized ssDNA probes or by using biotin-tagged target oligonucleotides bound to avidin-modified nanoparticles on the sensor surface. However, simultaneous analysis of multiple analytes with QCM is not possible for multiplex applications on the same sensor surface.

### 3.7. Magnetic separation techniques combined with nanostructures

Magnetic separation (MS) has been traditionally used for the detection of various analytes coupled with different detection methods. In this technique, magnetic particles are coated with ligand molecules to capture the target and remove from solution by applying a magnetic field. Immunomagnetic separation (IMS) with antibodies (recognition agents) has been used for the detection of various bacteria and toxins (71, 134-138).

In the past decade, using magnetic NPs, instead of micrometer-sized counterparts, has aroused great interest, due to their high surface/volume ratio that offers more contact area for capture (139). El-Boubbou et al. (140) demonstrated that the avidity of mannosere coated magnetic NPs to concanavalin A (a mannos binding lectin) is 200-fold higher than the affinity of the monomeric mannos. In their study, magnetic glycol-NPs were used for the detection of *E. coli* followed by decontamination of the medium by removing 88% of the target bacteria. Vancomycin, an antibiotic which binds to D-Ala-D-Ala moieties on the cell walls of Gram-positive bacteria, was used to capture bacteria using biofunctional magnetic NPs (75, 139-141). In these studies, Lin’s group (75) used vancomycin-modified magnetic NPs to capture and concentrate bacteria. The isolated cells were characterized by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Magnetic separation reduced the interference of impurities in the mass spectra and allowed the detection of *Staphylococcus saprophyticus* and *Staphylococcus aureus* at a concentration of $7 \times 10^3$ cfu mL$^{-1}$. Similarly, vancomycin coated magnetic NPs were used to capture bacteria and after the separation from the rest of the sample, the isolated bacteria were stained by the vancomycin-fluorescein amine conjugate for analysis with a fluorescence microscope (141). A rapid (2 h) and sensitive (10 cfu mL$^{-1}$) detection of *E. coli* and coagulase-negative *Staphylococcus* in blood samples was achieved with this approach. Liu et al. (142) used pigeon ovalbumin coated magnetic NPs to probe *Pseudomonas aeruginosa* from complex samples and the trapped bacterial cells were characterized by MALDI-MS analysis. Although the developed assay provides rapid detection of bacteria, the limit of detection was not specified in the study.

Magnetic NPs have also been used for developing rapid and selective methods to detect bacteria in food samples. Amagliani *et al.* (143) demonstrated a novel method using magnetic NP-based isolation of bacterial DNA directly from milk with PCR analysis. *L. monocytogenes* was detected with a sensitivity of 10 cfu mL$^{-1}$. Another study for the detection of *L. monocytogenes* in milk samples was performed with immunomagnetic separation (IMS) using NPs with covalently bound antibodies (144). The NP-based IMS in combination with real-time PCR provided detection of $\geq 1 \times 10^6$ cfu mL$^{-1}$. *L. monocytogenes* in milk. Antibody-magnetic NP conjugate was also used for the detection of *E. coli* O157:H7 in ground beef samples (70). Enumeration of the captured bacteria was exhibited with interdigitated array microelectrode and the lowest detection limits of *E. coli* O157:H7 in food was $8.0 \times 10^2$ cfu mL$^{-1}$. Ravindranath *et al.* (145) isolated two different bacteria species from food matrices by using anti-*E. coli* O157:H7 and anti-*Salmonella typhimurium* (*S. typhimurium*) antibody conjugated magnetic NPs. The captured pathogens were detected by label free infrared fingerprinting with a detection limit of $10^3-10^4$ cfu mL$^{-1}$. Although, the sensitivity of the developed method was lower than other methods, the procedure was completed in less than 30 min. On the otherhand, a rapid and sensitive method for the detection of *E. coli* was developed by combining immunomagnetic NP separation with ATP bioluminescence (19). With this method, *E. coli* was detected in milk samples in 1 h with LOD of 20 cfu mL$^{-1}$ which is one of the most sensitive processes reported for *E. coli* to date.

In summary, magnetic NPs provide higher capturing efficiency compared with micro-sized magnetic particles, due to their high surface area. This increased efficiency of magnetic separation leads to more sensitive and rapid detection of microorganisms. However, most of the studies used highly expensive and inconvenient instruments for detection. So, efforts should be focused on...
3.7. Optical detection techniques combined with nanostructures

Nanomaterials, having different properties, have been employed as part of optical methods for improved performance. Fluorescence labeling techniques allow sensitive detection of various analytes. However, low photostability and low intensity of traditional fluorophores paved the development of novel fluorescence labels. Dye-doped NP technology provides high intensity and photo stability by encapsulating many thousands of dye molecules in a protective silica matrix (146). Dye-doped NPs coupled with antibodies have been used in a variety of applications, including fluorescence-linked immunosorbent assay (FLISA), immunocytchemistry, immunohistochemistry, DNA, and protein microarrays (146). Zhao et al. (147) demonstrated a bioassay based on dye-doped silica NPs conjugated with antibodies combined with both spectrofluorometer and flow cytometry measurements. The developed assay enabled the detection of single E. coli O157:H7 cell in less than 20 min and the detection limits of the assay in ground beef samples were found to be between 1 and 400 bacterial cells.

QDs, semiconductor nanocrystals in the size range of 1-10 nm, are highly luminescent fluorophores with broad excitation and narrow emission spectra. The high chemical and photo stability and size-dependent photoluminescence properties of QDs make them ideal labels for biological applications (148). Ji et al. (149) developed a biosensor for the detection of paraaxon, an organophosphorus compound and the active metabolite of the insecticide parathion, using QDs and an organophosphorus hydrolase bioconjugate. The photoluminescence intensity of the organophosphorus hydrolyase-QD conjugate was quenched as a function of photoluminescence intensity of the organophosphorus compound and the active metabolite of organophosphorus hydrolase bioconjugate. The working range was also established at 0-10^7 cfu mL^-1. IMS coupled with QD labeling allowed the detection of E. coli in water samples at 8.9x10^5 and 1.9x10^6 cfu mL^-1 (155). Although, similar methods were used in these studies, a lower detection limit was achieved in the latter due to the optimization of both the IMS and QD concentration.

The tunable emission property of QDs provides simultaneous detection of different targets in the same sample. Goldman et al. (156) reported the simultaneous detection of four different toxins using antibody-QD conjugates with different colors. In this study, wells of a microtiter plate were coated with antibodies against cholera toxin, ricin, shiga-like toxin 1, and SEB that are commonly responsible for food and water borne-illnesses (156). After capturing the target toxins in the wells, QD conjugated secondary antibodies were added to label the toxins. Quantification of the four toxins was performed with fluorometric measurement. Coupling IMS with multicolor QD conjugated secondary antibodies was used for the simultaneous detection of E. coli and S. typhimurium with a detection limit of 10^2 cfu mL^-1 (154). In a similar approach, E. coli and S. enteritidis were detected simultaneously by coupling IMS with QD label with a lower detection limit of 5x10^2 cfu mL^-1 and 4x10^2 cfu mL^-1 for E. coli and for S. enteritidis, respectively (138). The enhanced sensitivity of the assay was probably due to the optimization of the primary antibodies used in IMS, the ratio of QDs to antibodies during the conjugation and the concentration of QD-antibody conjugates used in labeling the cells. Elimination of quenching between magnetic beads and QDs by separating QDs from complex after the labeling process may also have attributed to the enhanced sensitivity of this method.

In fluorescence immunoassays, QD-antibody bioconjugates were used to detect or image various analytes (150). Hahn et al. (151) utilized QDs to develop an indirect fluorometric assay for the detection of E. coli O157:H7. In this study, bacterial cells were first conjugated with biotinylated antibodies and then, streptavidin coated QDs were added to label the bacteria via streptavidin-biotin interaction. Although the sensitivity of the assay was increased using indirect labeling with QDs in place of organic dyes, the detection limit was not lower than 10^5 cfu mL^-1. Dwarakanath et al. (152) demonstrated that QD-antibody or QD-DNA aptamer conjugates exhibited fluorescence emission with blue shifts due to the binding of E. coli O111:B4, S. typhimurium, and B. subtilis spores. Although the assay was not applied to real sample analysis, the results obtained were promising novel, rapid, and convenient for bacteria detection in food.

Coupling IMS technique with QD labeling provided the detection of microorganisms with lower detection limits. A fluorometric detection method for E. coli O157:H7 was demonstrated by capturing the bacteria with antibody conjugated magnetic beads and biotin-conjugated secondary antibodies to form sandwich immune complexes that were later labeled with QDs via biotin-streptavidin conjugation (153). The enumeration of bacteria was achieved for 10^3-10^4 cfu mL^-1 in less than 2 h. Yang and Li (154) followed the same procedure for the detection of S. typhimurium in chicken carcass wash water and the working range was also established at 0-10^5 cfu mL^-1. IMS coupled with QD labeling allowed the detection of E. coli in water samples at 8.9x10^5 and 1.9x10^6 cfu mL^-1 (155). Although, similar methods were used in these studies, a lower detection limit was achieved in the latter due to the optimization of both the IMS and QD concentration.

Surface plasmon resonance (SPR) sensor, a type of optical sensor that measures the changes of the refractive index at the sensor surface, has been successfully used for the rapid and label-free detection of various analytes including toxins and microorganisms (138, 157-158). Utilizing AuNPs in SPR measurements provided enhancement of SPR signal during the binding of target molecules (159-160). Joung et al. (161) used cationic AuNPs for signal amplification by interacting these with E. coli 16s rRNA hybridized on the peptide nucleic acid probe immobilized on SPR sensor surface. The detection limit of the developed method was 58.2 pg mL^-1 for E. coli rRNA.
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which was approximately 5000-fold greater than the limit of the system without the AuNPs for signal enhancement.

Localized surface plasmon resonance (LSPR) sensor, which relies on the oscillation of free electrons that occurs when light is incident on noble-metal NPs, allows sensitive detection of target molecules. The sensitivity of the LSPR sensors can be improved by modifying the size and shape of the nanoparticles. LSPR immunosensors provided rapid and label-free detection of analytes when capture antibodies were bound to the NPs (162). Zhu et al. (163) developed a LSPR biosensor using triangular hybrid Au-Ag NPs covalently bound to SEB antibody and the detection sensitivity of SEB was found to be at ng mL⁻¹ levels. LSPR-based casein immunosensor was developed using Au-capped silica NPs for the detection of casein in milk samples with a detection limit of 10 ng mL⁻¹ (164). Although, LSPR biosensors are promising for detection of small analytes in food samples, the detection of bacteria by LSPR sensors are limited by the plasmon peak shift induced by the binding of the bacteria (165).

The unique chemical and physical properties of NPs make them promising candidates for constructing novel sensing devices. A variety of NPs, such as metal, metal oxide, composite, and semiconductor NPs have been considered. AuNPs are one of the most popular nanomaterials for sensing due to their size-dependent electronic and optical properties. The unique properties of AuNPs that include physical stability, electrical conductance, and their optical properties make them promising candidates in the development of sensors. They have been successfully applied in label technology because of their controllable-size distribution, long-term stability, and biocompatibility with antibodies, antigen proteins, DNA etc. (166). AuNP-based bio-bar-code amplification (BCA) assays have been reported to be highly sensitive and capable of detecting proteins (i.e. prostate-specific antigen (as low as 30 aM and human immunodeficiency virus type 1 p24 antigen at LOD of 0.1 pg mL⁻¹) and pathogens (S. enteritidis and B. anthracis). Zhang et al. (167) reported a highly amplified bio-barcode DNA assay for the rapid detection of the insertion element (Iel) gene of S. enteritidis. In this study, they used Au-NPs which were coated with the target-specific DNA probe and fluorescein-labeled barcode DNA to form a sandwich structure. The bio-barcode DNA was released from the AuNPs for LOD as low as 2.15 x 10⁻¹⁶ mol (or 1 ng mL⁻¹). In a similar study, Zhang et al. (168) developed a highly amplified nanoparticle-based, bio-barcoded electrochemical biosensor for the multiple detection of pagA gene from B. anthracis and insertion element (Iel) gene from S. enteritidis by using AuNPs, magnetic NPs and NP tracers (NTs) such as PbS and CdS. The sensitivity of the sandwich type biosensor was 0.2 pg mL⁻¹ for single detection of pagA gene from B. anthracis using PbSNTs and 0.5 ngmL⁻¹ and 50 pg mL⁻¹ for multiple detection of the insertion element (Iel) gene of S. enteritidis using CdS and pagA gene from B. anthracis using PbS NTs, respectively (168).

AuNPs are also used as Raman labels to enhance Surface-enhanced Raman scattering (SERS) signal intensity. SERS has been used to obtain molecular information through sharp and easily distinguishable vibrational bands. This information is commonly used for detection of (bio)molecules (169). Since the introduction of SERS, nanomaterials are of significant interest as SERS substrates due to their tunable optical properties. The design and fabrication of a variety of nanomaterials with large SERS enhancement factor have been reported. The SERS technique provides 10⁵-10¹⁴-fold enhancement in Raman signal intensity, and this is sufficient to detect picomolar to femtomolar amounts of biomolecules (170-172). SERS substrates constructed from Au and AgNPs have been used to detect microorganisms (172-173), protein (174), and nucleotides (175). Anisotropic metallic NPs such as Au or Ag have been demonstrated as novel SERS tags for immunoassay. The results suggested that SERS tags could be used for multiplex and ultrasensitive detection of biomolecules. More specifically, Au nanorods have been shown to have distinct optical properties that depend on their shape. In particular, they have two plasmon absorption bands, one the transverse plasmon (TP) band, and the longitudinal plasmon (LP) band. The high-energy band corresponds to the oscillation of the electron perpendicular to the rod axis (TP) and the other absorption band, which is red shifted to lower energies caused by the oscillation of electrons along the rod axis (LP). The position and intensity of these bands can be affected by changes in the dielectric constant around the vicinity of NPs. The elongated NPs have also higher sensitivity to the local dielectric environment and observed SERS signal intensity is substantially increased (176).

A variety of Au nanorod particle different aspect ratios with numerous surface functionalities can be easily synthesized (177) and used as SERS substrates (178). Yazgan et al. (179) quantitatively measured the proteolytic enzyme activity using the SERS probe with a LOD of 0.43 mU mL⁻¹ in less than 40 min. In another study, Temur et al. (43) used two different NPs to detect E. coli in water samples using SERS with a reported the LOD as 4 cfu mL⁻¹.

UV resonance Raman (UVRR) spectroscopy was also used to get reproducible acquisition of information rich Raman fingerprint (180). An aggregated Ag colloid substrate was also used to analyze pathogens such as E. coli, Klebsiella oxytoca, Klebsiella pneumoniae, Citrobacter freundii, Enterococcus spp., and Proteus mirabilis (181). While each spectrum took 10 s to collect, to acquire reproducible data, 50 spectra were collected making the spectral acquisition times per bacterium approximately 8 min. The multivariate statistical techniques of discriminant function analysis and hierarchical cluster analysis were applied in order to group these organisms based on their spectral fingerprints. Utilizing the whole-organism ‘fingerprints’ obtained by UVRR they were able to discriminate successfully between UTI pathogens using chemometric cluster analyses. The resultant ordination plots and dendrograms showed correct groupings for these organisms, including discrimination to strain level for a sample group of E. coli. However, one of the main drawbacks of the technique for samples with
### Table 1. Some applications of nanostructures for detection of food components and contaminants

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Nanostructure</th>
<th>Target</th>
<th>LOD</th>
<th>Analysis time</th>
<th>Food matrix</th>
<th>Referenc e</th>
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<tr>
<td>Amperometry</td>
<td>Pt-NP modified CNT</td>
<td>Glucose</td>
<td>NA</td>
<td>5 sec</td>
<td>NA</td>
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<tr>
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<td>NA</td>
<td>NA</td>
<td>91-104</td>
</tr>
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<td>Amperometry</td>
<td>Au NP</td>
<td>Glucose, titania nanotubular electrode</td>
<td>0.1 mM</td>
<td>3 sec</td>
<td>NA</td>
<td>105</td>
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<tr>
<td>Amperometry</td>
<td>Pt NP decorated CNT</td>
<td>Glucose</td>
<td>5.7 μM</td>
<td>3 sec</td>
<td>NA</td>
<td>106</td>
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<tr>
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<td>CNT/ Pt NP</td>
<td>Glucose</td>
<td>6.7 mM</td>
<td>5 sec</td>
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<td>Amperometry</td>
<td>SiO₂ coated Fe₃O₄ magnetic NP</td>
<td>Glucose</td>
<td>800 nM</td>
<td>NA</td>
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<tr>
<td>Amperometry</td>
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<td>H₂O₂ and nitrite</td>
<td>2.4 x 10⁻⁴ and 6.5 x 10⁻⁵ M</td>
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<td>NA</td>
<td>109</td>
</tr>
<tr>
<td>Amperometry</td>
<td>Pt NP decorated CNT</td>
<td>E. coli</td>
<td>10 cfu mL⁻¹</td>
<td>NA</td>
<td>Water</td>
<td>184</td>
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<tr>
<td>Amperometry</td>
<td>Pt NP coated gold nanoporous film</td>
<td>E. coli</td>
<td>10 cfu mL⁻¹</td>
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<td>NA</td>
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<td>Salmonella spp. ssDNA</td>
<td>9 fmol</td>
<td>NA</td>
<td>NA</td>
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<td>Conductometry</td>
<td>Polyamline coated magnetic NPs</td>
<td>B. anthracis endospores</td>
<td>4.2 x 10⁶ cfu mL⁻¹</td>
<td>16 min</td>
<td>Romaine lettuce, whole milk, and ground beef</td>
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<tr>
<td>Conductometry</td>
<td>Nanogold/chitosan-multi-walled CNT</td>
<td>SEB</td>
<td>0.5 ng mL⁻¹</td>
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<td>NA</td>
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<tr>
<td>Voltammetry</td>
<td>Au NP/CNT hybrid</td>
<td>Human IgG</td>
<td>40 pg mL⁻¹</td>
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<td>Pesticide sensitivity test</td>
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<td>Voltammetry</td>
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<td>DNA</td>
<td>15 nM</td>
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<td>Voltammetry</td>
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<td>Voltammetry</td>
<td>NP encoded beads (ZnS, CdS, PbS)</td>
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<td>NA NA</td>
<td>NA</td>
<td>NA</td>
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<td>Impedance Spectroscopy</td>
<td>Magnetic NPs</td>
<td>E. coli O157:H7</td>
<td>7.4 x 10⁶ cfu mL⁻¹</td>
<td>35 min</td>
<td>Ground Beef</td>
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<td>Impedance Spectroscopy</td>
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<td>Impedance Spectroscopy</td>
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<td>DNA</td>
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<td>Impedance Spectroscopy</td>
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<td>Goat anti-fluorescein</td>
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<td>IgG</td>
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<td>ECL</td>
<td>Au NPs</td>
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<td>Au NPs</td>
<td>Lysosome</td>
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<td>QDs</td>
<td>Acetylcholine esterase inhibitors</td>
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<td>Cytochrome c</td>
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<td>2 h</td>
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<td>Fluorescent microscopy</td>
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<td>10⁸ cells mL⁻¹</td>
<td>2 h</td>
<td>NA</td>
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<td>S. saprophyticus S. aureus</td>
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<td>Urine sample</td>
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<td>Fluorescent microscopy</td>
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<td>2 h</td>
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<td>10 cfu mL⁻¹</td>
<td>7 h</td>
<td>Milk</td>
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<td>Real time-PCR</td>
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<td>L. monocytogenes</td>
<td>10⁶ cfu/0.5 mL</td>
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<td>Milk</td>
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<td>Interdigitated array microelectrode</td>
<td>Magnetic NPs</td>
<td>E. coli O157:H7</td>
<td>8 x 10⁶ cfu mL⁻¹</td>
<td>35 min</td>
<td>Ground beef</td>
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<td>IR fingerprinting</td>
<td>Magnetic NPs</td>
<td>E. coli O157:H7 S. typhimurium</td>
<td>10⁷-10⁸ cfu mL⁻¹</td>
<td>30 min</td>
<td>2% milk, Spinach extract</td>
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<td>ATP bioluminescence</td>
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<td>E. coli</td>
<td>20 cfu mL⁻¹</td>
<td>1 h</td>
<td>Milk</td>
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<tr>
<th>Spectrofluorometer</th>
<th>Dye-doped silica NPs</th>
<th>E. coli O157:H7</th>
<th>1 cell</th>
<th>20 min</th>
<th>Ground beef</th>
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<td>10^6 M</td>
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<td>10^4 cfu mL^-1</td>
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<td>NA</td>
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<td>S. typhimurium</td>
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<td>Chicken carcass wash water</td>
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<td>Hybrid Au-AgNPs</td>
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<td>Au capped silica NPs</td>
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<td>E. coli</td>
<td>10^1 cfu mL^-1</td>
<td>70 min</td>
<td>Water samples</td>
<td>43</td>
</tr>
</tbody>
</table>

SERS has been the inability to acquire a region of the sample matrix where both the SERS substrate and biomass were present (182). The selectivity and discrimination of the proposed technique was achieved by using an appropriate antibody.

Optical nanoparticles provide new perspectives in developing bioanalytical methods. Especially, nanoparticles used as fluorescent labels, such as dye-doped NPs and QDs, leads more sensitive detection methods due to their improved optical properties. In addition, QDs, having broad excitation and narrow emission properties, provides the multiplexed detection of various analytes. Nanoparticles are not only used as labels in optical detection methods, but also elicit the construction of novel sensing devices, such as LSPR and SERS, which promises rapid and sensitive detection.

4. CHALLENGES AND PROSPECTS

Recent studies on nanotechnology-based bioassays show promise for the development of rapid and sensitive detection methods for food components and contaminants. Nanomaterials such as metal and semiconductor NPs were used for signal enhancement in optical and electrochemical biosensors that improved the detection limit and eliminated the enrichment steps in bioassays. Developing a biosensor to detect single cell by combining NPs with techniques like flow cytometry and LSPR continuous to be a greater challenge for researchers. Using QDs as fluorescent labels in biological applications provided multiplexed detection of various analytes. The nanotechnology offers a simultaneous detection of multiple contaminants in a short time and has the potential to revolutionize food safety and save lives through prevention of food poisoning epidemics. Nanostructures offer advantages for detection of food pathogens and contaminants such as rapidity, low cost, and ease of use especially in aqueous food matrices.

Although a remarkable success has been made in using nanomaterials for the detection of food contaminants, toxins and pathogens, only a few studies focused on real food samples that are vital for the acceptance of nano-based sensors and assays in food analysis. Currently, difficulty in the synthesis of nanomaterials and the use of expensive instruments for the assays are obstacles for commercialization. Further research should be focused on the improvement of cost-effective and rapid assays with high sensitivity, accuracy, and precision by working on the design of new nanomaterials and the optimization of the integration of these nanomaterials with transducers. We believe that on-going improvements such as coupling with other techniques, such as IMS and/or SERS, etc. will make the use of NPs even more attractive and useful in the near future.

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