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Emerging Trends of Immunosensors Development for Detection of Food Toxins

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ABSTRACT

The present study highlights the ongoing threat of foodborne illnesses to public health, primarily caused by bacterial pathogens. Despite advancements in conventional microbiological testing techniques, which are sensitive but time-consuming, challenges remain in ensuring timely detection of contaminants throughout the food supply chain. The Hazard Analysis Critical Control Point (HACCP) system is recognized as a more effective approach to ensuring food safety, emphasizing proactive identification and control of hazards at critical points in production. Emerging technologies like quantitative polymerase chain reaction (PCR) and biosensors offer faster and more accurate detection methods, although with certain limitations. Biosensors such as ELISA, SPR, and electrochemical immunosensors, in particular, show promise due to their high sensitivity and specificity, enabling rapid detection of a wide range of contaminants. This paper underscores the importance of integrating advanced technologies with established food safety protocols to enhance the safety and quality of food products, benefiting consumers, producers, and regulatory agencies alike.

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Introduction

Foodborne poisonings continue to pose a threat to public health. Food can spread more than 200 recognized diseases (Gizaw, 2019). A wide range of viruses, fungi, heavy metals, chemicals, parasites, bacteria, and bacterial toxins are among the foodborne pathogens; however, the most common type of poisoning is caused by bacteria (Table 1). Less than twenty distinct bacteria are the originators. More than 90% of food poisonings linked to recognized pathogens occur each year and are caused by *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and enteropathogenic *Escherichia coli* (Ahuja et al., 2023; Elbehiry et al., 2023; Franco-Duarte et al., 2019). According to Moises (2009) and the Department of Health and Human Services (2012), these bacteria are primarily found in raw foods (Department of Health and Human Services, 2012.; Moises, 2009).

Widespread solutions throughout the entire feed and food chain-farm, transport, supply, and consumption—are required to safeguard consumers from pathogen ingestion, since food safety concerns consumers, food producers, and regulatory agencies (Moises, 2009). To ensure food safety and quality, it is important to analyze food items for the presence of both biological (pathogenic microorganisms) and chemical pollutants. Viable bacterial cells in food are isolated and counted using particular microbiological media in conventional bacterial testing techniques. Because these conventional techniques rely on the ability of microorganisms to proliferate and form visible colonies, they are very sensitive, low-cost, and take several days to yield results. For instance, it takes more than 4-5 days to detect Salmonella germs using the current official approach (ISO/DIS 6579, 2001) which entails a number of laborious sequential culture stages.

Table 1. Various Food Toxins from different respective Sources (Ahuja et al., 2023)

Class	Toxin's name	Source	Effect
Mycotoxins	Aflatoxin	*Aspergillus flavus* and *A. parasiticus*	Liver failure, cirrhosis
	Lysergic acid (ergot alkaloids)	*Claviceps purpurea*	Ergotism, vasoconstriction, uterine contraction
	Fumonisins B1 and B2	*Fusarium verticillioides* and *Fusarium proliferatum*	disruption of sphingolipid metabolism, leukoencephalomalacia
	Ochratoxin A	Aspergillus and Penicillium	Carcinogenic, immunotoxic mutagenic,nephrotoxic, and teratogenic
	Patulin	Aspergillus, Byssochlamys and Penicillium	Teratogenic, carcinogenic and mutagenic
	Zearalenone	*Fusarium graminearum, F. culmorum, F. crookwellense, F. poae, F. semitectum,* and *F. equiseti*	Hepatotoxicity, immunotoxicity, reproductive Toxicity
	Tentoxin	Alternaria	Genotoxic, mutagenic, and carcinogenic
Bacterial toxins	Cholera toxins	*Vibrio cholera*	Diarrhea
	Enterotoxins	*Staphylococcus epidermidis*	Toxic shock syndrome
	Shiga toxins	*Escherichia coli*	Gastrointestinal complications
	Botulinum toxins	*Clostridium botulinum*	Neurotoxic
	Cereulide	*Bacillus cereus*	Dysfunction of liver, pancreatic islet, intestines, brain,
Marine biotoxins	Saxitoxin	Cyanobacteria and dinoflagellates	Neurotoxin, paralysis
	domoic acid	Diatoms	Neurotoxin
	Azaspiracid	Azadiniumpoporum	Diarrheic shellfish poisoning
	Brevetoxin	*Karenia brevis*	Immunotoxicity
	okadaic acid	*Halichondria melanodocia* and *Halichondria okadai*	Diarrhea, nausea
Plant-based toxins	Cyanogenic glycosides	Almonds, cassava, pome fruit, stone fruit	Tissue damage
	Furocoumarins	Citrus fruits	Skin cancer
	Ptaquiloside	*Bracken ferns*	Carcinogenic
	Dehydropyrrolizidine	Cyanoglossum, Senecio, Echium, Crotalaria, Heliotropium, Symphytum, Trichodesma	Carcinogenic

According to Moises (2009) and Ricci (2007), these procedures generally consist of the following: (a) pre-enrichment; (b) selective enrichment; (c) isolation; and (d) biochemical and serological confirmation if presumed positive Salmonella colonies form (Moises, 2009; Ricci, 2007).

Instead of using process control, product testing has been the primary method used to ensure food safety over the last ten years. The primary issue with end-product testing is the large number of samples that must be analyzed in order to determine if a product batch is safe, particularly in cases where it is anticipated that contaminants will be spread unevenly across the batch. Moreover, end-product testing does not pinpoint the reasons behind failures—rather, it merely finds them (Ricci, 2007). It is now widely acknowledged that the HACCP (Hazard Analysis Critical Control Point) system is the most efficient way to guarantee food safety. It does this by identifying certain dangers and providing controls for them. Rather than depending solely on evaluating the finished product, HACCP can be implemented at every stage of the food chain, from primary manufacturing to ultimate consumption. Applying HACCP can improve food safety and help regulatory agencies with their inspections (Moises, 2009; Ricci, 2007; Weinroth et al., 2018).

Quantitative polymerase chain reaction (PCR) could be an accurate, fast, specific, and sensitive method for detecting small amounts of pathogen deoxyribonucleic acid (DNA) in food samples. Customary microbiological methods, such as cell culture techniques, are often laborious and ineffective due to their incompatibility with the production chain's speed and food distribution, its endurance, and operational costs (Ahuja et al., 2023; Vidic et al., 2019; Vinayaka et al., 2019). Furthermore, bacterial strains can fail regular growth processes and lead to false analysis results. Unfortunately, DNA-based assays can only detect the presence of toxin-producing organisms and do not quantify the amount of effective toxins. Online detection with PCR methods is also expensive and requires well-trained personnel (Ahuja et al., 2023; Moises, 2009; Paul Leonard, 2003).

Standard techniques in instrumental analytical chemistry, like mass spectrometry, liquid chromatography, infrared, or UV/Vis spectrometry, are effective in precisely determining pathogens. However, they need much time to prepare samples and are typically not portable, making them unsuitable for online monitoring, like in the production process (Ahuja et al., 2023; Franco-Duarte et al., 2019). On the other hand, sensor-based bioassays and microarray techniques are quick and sensitive methods for

online detection and automated process control in the food production and supply chain (Calabria et al., 2022; Mazur et al., 2023; Moises, 2009; Rahimi et al., 2020; Ricci, 2007; Saini et al., 2021). The use of the HACCP system for process line management at Critical Control Points, or CCPs, has raised the need for quick, sensitive, and precise ways to identify chemical and biological contaminants in the process. Tests that may be finished in minutes or hours, for example, would allow processors to act quickly to remove pollutants when found. For this reason, portable, quick, and sensitive biosensor technology must be developed (Ricci, 2007). Biosensors are significant because of their high sensitivity and specificity, which enable little sample pre-treatment and the detection of a wide range of analytes in complicated samples. A biosensor is an analytical tool that combines a transducer that generates a signal proportional to the target analyte concentration with the selectivity of a biological interaction. A signal transducer (such as optical, amperometric, potentiometric, and acoustic) connected to a data acquisition and processing system is in close proximity to the biological recognition element (e.g., antibodies, enzymes, nucleic acids, animal or vegetable tissues, receptors, and microbial cells) (Baranwal et al., 2022; Paul Leonard, 2003; Ricci, 2007; Walper et al., 2018). A device is referred to as an immunosensor when it uses antibodies or antibody fragments as a molecular recognition element to detect specific analytes (antigens) to form a stable complex (Ricci, 2007; Tokarskyy & Marshall, 2008). Immunosensors can be categorized into four main groups based on how they transduce signals: electrochemical, optical, piezoelectric, and thermometric (Paul Leonard, 2003; Ricci, 2007).

An external alternating electric field is used to induce resonance in the transducing materials of piezoelectric immunosensors, which include bulk acoustic and surface acoustic wave sensors. The concentration of the target analyte is correlated with the mass variation caused by antigen-antibody binding on the surface of the quartz crystal. Potentiometric and amperometric immunosensors make up the majority of electrochemical immunosensors. The basic principle of potentiometric immunosensors is the detection of a shift in potential brought about by antigen-antibody interactions on the sensor surface. The current produced when electroactive species are either reduced or oxidized at the electrode at a fixed potential is measured in amperometric immunoassays. Fiber optic and evanescent wave biosensors are examples of optical immunosensors. Their operation relies on measuring how much light the immunoreactants absorb or emit. Refractive index, polarization, absorbance, and luminescence changes are some metrics that can be used to quantify the interactions between light and the immunoreactants. SPR is a very appealing optical-signal transducer that makes it possible to monitor biochemical interactions in real time without having to label reagents (Dhesingh Ravi Shankaran, 2007).

The majority of developed immunosensors are based on sandwich or competitive assays, which are used to detect high molecular weight molecules (like proteins and cells) and low molecular weight molecules (such as herbicides and toxins), respectively. For the development of competitive immunosensors, one of two methods can be used: (1) the labeled antigen (Ag*) competes with the unlabeled antigen (Ag) (present in the sample) for binding to the antibody (Ab) immobilized on the sensor surface (Figure 1A-C), or (2) the unlabeled antigen (Ag) (the analyte to be detected) competes with a labeled secondary antibody (Ab*) or a labeled antigen mimic for binding to the immobilized primary antibody (Ab) on the sensor surface (Figure 1D-E). Typically, antigens—especially those with small molecular weights—are conjugated with a protein (e.g., Ag–BSA, Ag–KLH, Ag–OVA) to facilitate immobilization and antibody interaction. Direct competitive immunoassay is a description of both of these methods (Ricci, 2007). The second format is typically chosen because it prevents all of the issues associated with antibody immobilization, such as loss of affinity and improper antibody orientation. It can also be used in situations where enzyme-conjugated primary antibodies are unavailable for the selected analyte. This format is known as indirect competitive immunoassay (Figure 1E), and it involves the use of an anti-species IgG-enzyme, also known as a secondary antibody, as a label after it has bound with the Fc region of the primary antibody. Following the interaction of immobilized antibodies (Ab) with free antigens in a sandwich assay, labelled antibodies (Ab*) that target a second antigen binding site are added, resulting in Ag being sandwiched between two antibodies (Ab and Ab*) Figure 1B. Transducers that enable label-free detection and direct quantification of the immunocomplex (Ab–Ag) include modern optical sensors based on surface plasma resonance (SPR) (Surface Plasma Resonance) and piezoelectric sensors (quartz crystal microbalance, QCM) (Paul Leonard, 2003; Ricci, 2007).

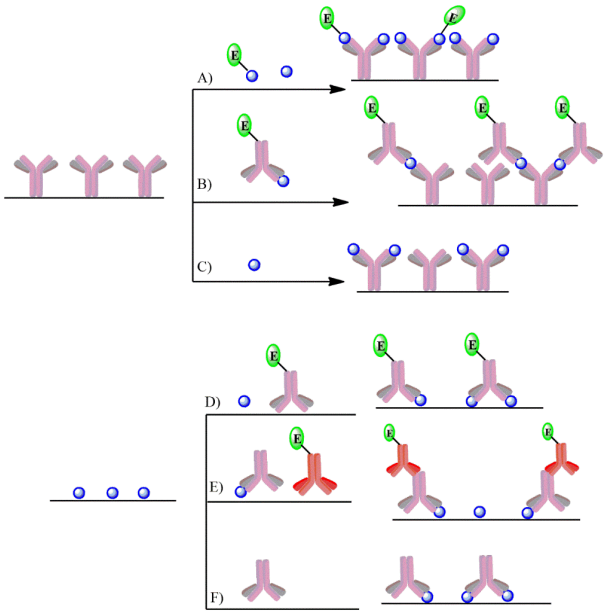

Figure 1. Forms for immunoassays. (Top) An immobilized antibody (Y) on the support, three different assay methods are available: (A) direct competitive assay with an enzyme-labeled antigen; (B) sandwich assay with an enzyme-labeled detection antibody; and (C) direct assay (for SPR and QCM). (Bottom) Antigen (●) immobilized on the support: (D) direct competitive assay with an enzyme-labelled primary antibody; (E) indirect competitive assay with an enzyme-labelled secondary antibody. (F) Direct testing (QCM and SPR). A functional electrode supports electrochemical immunosensors, whereas a chip or quartz crystal is used for SPR and QCM. SPR and QCM measurements for competitive and sandwich assays (A, B, D) do not use labels (Ricci, 2007).

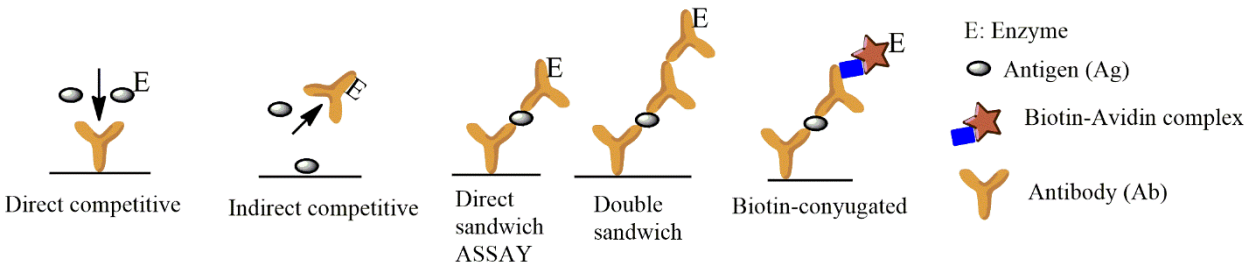

Figure 2. Different types of ELISA essay

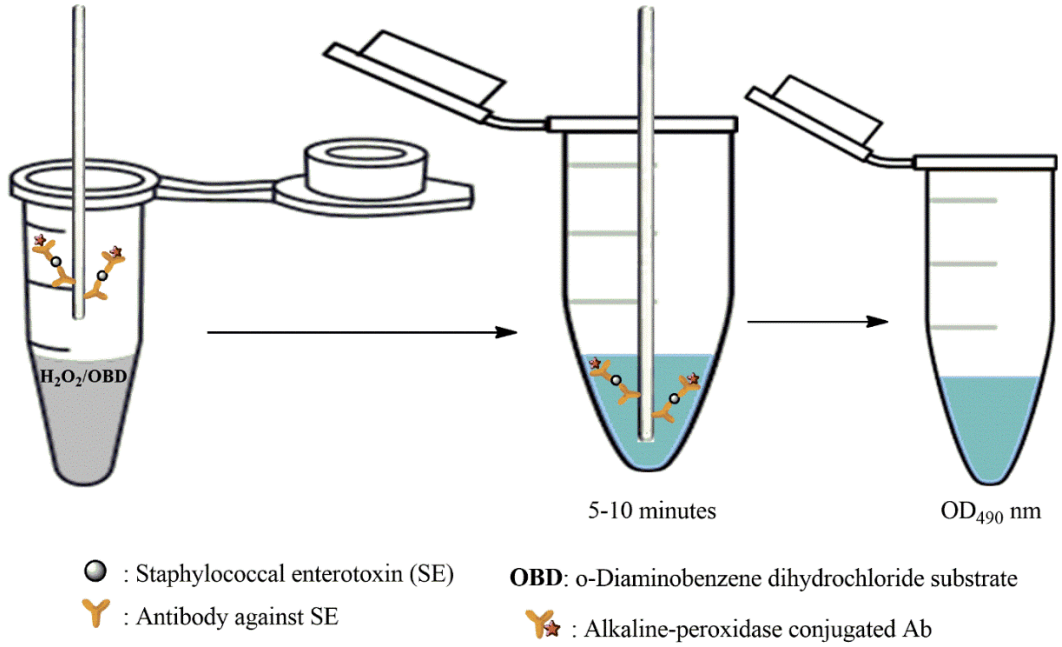

Figure 3. Dipstick sandwich assay scheme (Moises, 2009)

ELISA for Toxin Determination

Common instrumental analysis methods like gel electrophoresis, gas chromatography, mass spectrometry, and high-pressure liquid chromatography (HPLC) tend to be expensive, time-consuming, and require personnel with the necessary training to handle. Rapidity, specificity, sensitivity, multiplicity, and the availability of affordable, easily obtainable equipment are among the requirements for modern high throughput analysis that are satisfied by immunochemical techniques like Enzyme-Linked Immunosorbent Assay (ELISA). Additionally, portable instruments can be used to perform the entire analysis. Compared to more traditional instrumental techniques like quantitative HPLC, these benefits made ELISAs well-known, high-throughput assays with low sample volume requirements and frequently fewer involved sample preparation steps (Moises & Schaeferling, 2009; Serrano-Pertierra et al., 2020). The most prevalent toxin contaminations in food matrices have been the focus of numerous assays compiled in Table 2. A schematic overview of the fundamental ELISA formats is shown in Figure 2. The dipstick sandwich assay (Figure 3) is a variation of the traditional ELISA developed for identifying *Staphylococcal enterotoxins* (SEs). On a polystyrene stick dipped in homogenized cheese samples contaminated with SEB, capture antibodies for SEB are applied. The standard sandwich assay involves the addition of primary antibodies that have been coupled to horseradish peroxidase (HRP) afterwards. The assembly is put into a tube with O-diaminobenzene dihydrochloride and H_2O_2. When HRP is present, this substrate undergoes an enzymatic conversion to a blue reaction product. After 5 to 10 minutes of reaction time, the optical density of the solution can be measured at 490 nm using a commercial fiber-optic probe (Celine Morissette, 1991; G J Doellgast, 1993; Hou et al., 2023; Moises, 2009).

Solid phase immunoassays, such as the Enzyme-Linked Immunosorbent Assay (ELISA), are among the well-known methods that are frequently used for routine sample analysis because of their simple protocols, capacity to handle multiple samples, and automation. Even with these advantages, pathogen detection still requires a higher sensitivity of ELISA. Numerous reports in the literature have lowered the limits of detection for Salmonella spp. using ELISA techniques, but they call for extra procedures and supplies (Paniel & Noguer, 2019; Sruti Chattopadhyay, 2013). In order to pre-concentrate cells from mixed cultures, immunomagnetic microbeads coated with antibodies were used in conjunction with ELISA (IMS-ELISA); however, the detection sensitivity was comparable to that of conventional ELISA (10^5–10^6 CFU/mL) (Sruti Chattopadhyay, 2013; Yongcheng Liu, 2001). Liebana et al. (2009) reported a rapid detection of Salmonella from artificially inoculated milk using the same technique and a developed electrode.

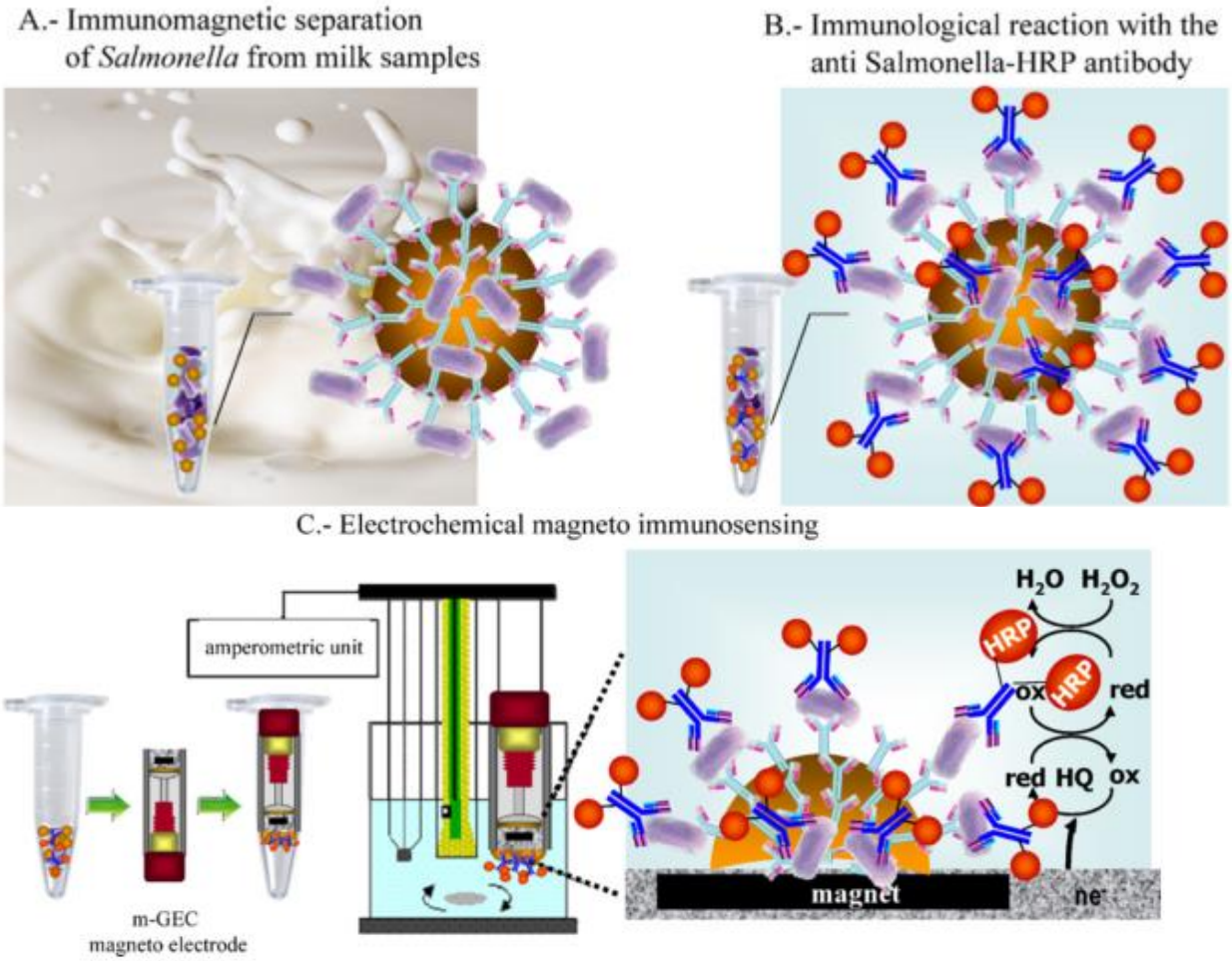

Figure 4. Diagram illustrating the IMS/m-GEC electrochemical immunosensor technique (Susana Liébana, 2009)

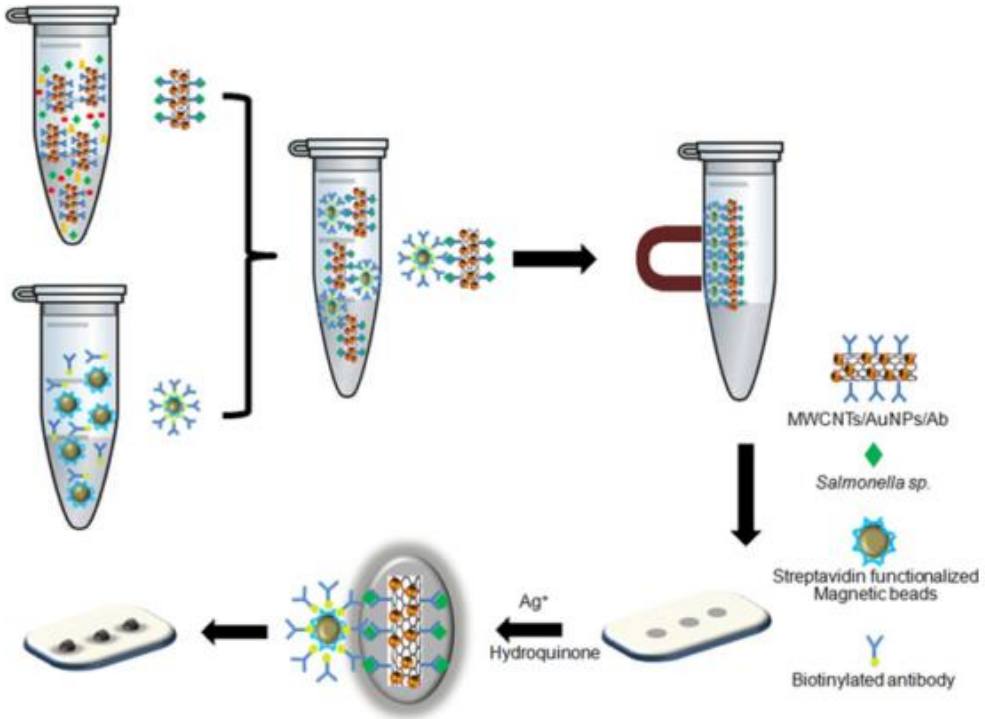

Figure 5. Schematic representation of S. enteric serovar typhimurium detection based on MWCNTs/AuNPs/Ab1 and MBs/Ab2 and signal amplified by silver reduction. (Moegiratul Amaro, 2012)

The detection limit was improved to ~7.5×10^3 cells/mL (Sruti Chattopadhyay, 2013; Susana Liébana, 2009). *Salmonella enterica* serovar Typhimurium was found to be sensitively detected by scano-magneto immunoassay employing multiwalled carbon nanotubes/gold nanoparticles (MWCNTs/AuNPs) in combination with an ELISA nanocomposite, with detection as low as 42 CFU/ml (Moegiratul Amaro, 2012; Tessaro et al., 2022).

Immuno-magnetic separation (IMS) holds promise as a practical method for removing food compounds from a sample and selectively enriching target bacterial cells using paramagnetic particles coated with particular antibodies. Food diagnostics uses magnetic separation techniques extensively these days. Microbiological tests on foods are more sensitive and to take less time overall when IMS techniques are used. The traditional ISO Salmonella technique was outperformed by IMS employing Dynabeads Anti-Salmonella (Dynal, Oslo, Norway) and plating. When compared to results obtained using traditional cultural methods, magnetic beads coated with an antibody against *Escherichia coli* O157 (Dynabeads Anti-E. coli O157, Dynal) may also increase the isolation rate of *E. coli* O157 (Frenea-Robin & Marchalot, 2022; M. Uyttendaele, 2000; Sauli et al., 2024; Yongcheng Liu, 2002).

The immunomagnetic separation assay (Figure 6) uses dispersed magnetic particles pre-coated with polyclonal capture antibodies specific for *C. perfringens* enterotoxin A. To perform the assay, target enterotoxins included in the sample were placed into microwell plates blocked with bovine serum albumin (BSA) and incubated therein with the specific antibody-coated magnetic particles. Other pathogens that are separated and detected using this method include the detection of *Staphylococcus aureus* thermostable nuclease in composite milk (Siamak P. Yazdankhah, 1999) and the separation and enrichment of *Alicyclobacillus spp.* in apple juice (Zhouli Wang, 2013).

Toxins were bound to the particles and then used a magnetic concentrator to draw them to the bottom of the microtiter plates. An enzyme antienterotoxin complex was constructed by adding a biotinylated enterotoxin-specific target antibody after the loaded particles had been concentrated and cleaned. A preformed avidin–biotin alkaline phosphatase complex (AP) or streptavidin–biotin HRP complex was added and incubated to guarantee quantitative complex formation. After particle concentration at the bottom of the wells using a magnetic concentrator, a p-nitrophenylphosphate or azinobenzthiozoline sulfonate substrate was used to quantify the amount of toxin in the sample. After moving the colored reaction mixture to a fresh microtiter plate, the absorbance at 414 nm was determined (Wu et al., 2016). The high endoprotease activity of Botulinum neurotoxins is used in sensitive assays. By cleaving various neuronal protein isoforms, these enzymes regulate how synaptic vesicles dock with the synaptic membrane. Clostridium botulinum neurotoxin B (BoNT B) in food samples can be detected with synthetic peptide substrates immobilized on a solid phase of a column. After being added to the column, the toxin cleaves the peptides, which are also biotinylated at the terminal position. Using particular antibodies conjugated to HRP, the eluate containing the released peptide fragment is transferred to microwell plates coated with streptavidin and detected. To determine the serotype A of botulinum neurotoxin, a straightforward dot blot immunoassay was created that may be used as a portable device and an automated screening tool ((Leka et al., 2023; Mechaly et al., 2022; Moises, 2009; Nguyen et al., 2024).

Several polymeric substrates have attracted interest because of their fascinating features, and they are utilized as a matrix to identify bacteria. Due to its exceptional mechanical and thermal properties, chemical resistance to the majority of solvents, good biocompatibility, and stability against bacteria, polyacrylonitrile (PAN) is one of these interesting biomedical materials. Furthermore, the nitrile groups on the surface act as interfaces for the multilateral modification of surface functionality in order to covalently immobilize biological entities. In the assay intended to remove non-specific binding, the generated functional groups allow the use of high concentrations of blocking agent in addition to covalent coupling (Sruti Chattopadhyay, 2013; Swati Jain, 2012). The different PAN forms—membrane, granules, strips, fibers, etc.—can be utilized as an immunoassay matrix. PAN fibers are a great option for immobilizing biomolecules because of their high surface area to volume ratio. Various enzymes, antibodies, and chemicals have been immobilized using reduced PAN fibers (Ali Khalafi-Nezhad, 2012; Iftikhar et al., 2023; Sruti Chattopadhyay, 2013). *Salmonella typhimurium*, a foodborne pathogen, has been detected with a very low detection limit of 10 cells/mL of bacteria thanks to the improvement of detection sensitivity of conventional ELISA using modified polyacrylonitrile fiber as an alternative matrix (Sruti Chattopadhyay, 2013).

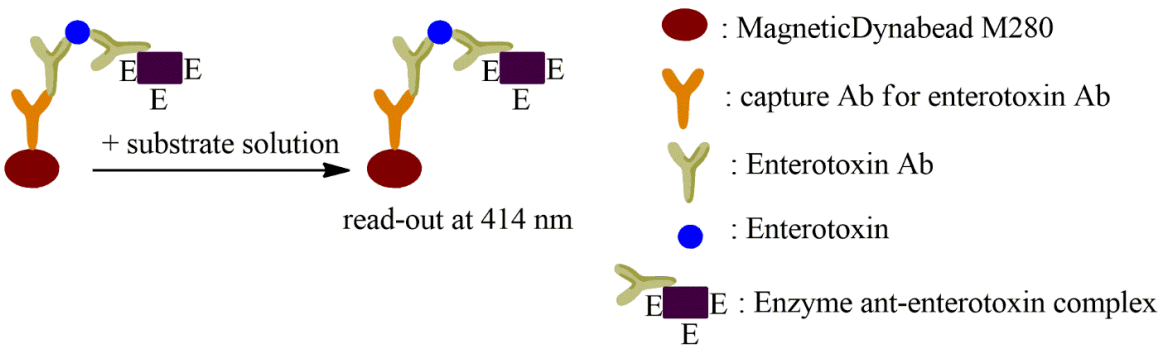

Figure 6. Immunomagnetic separation assay scheme (Moises, 2009)

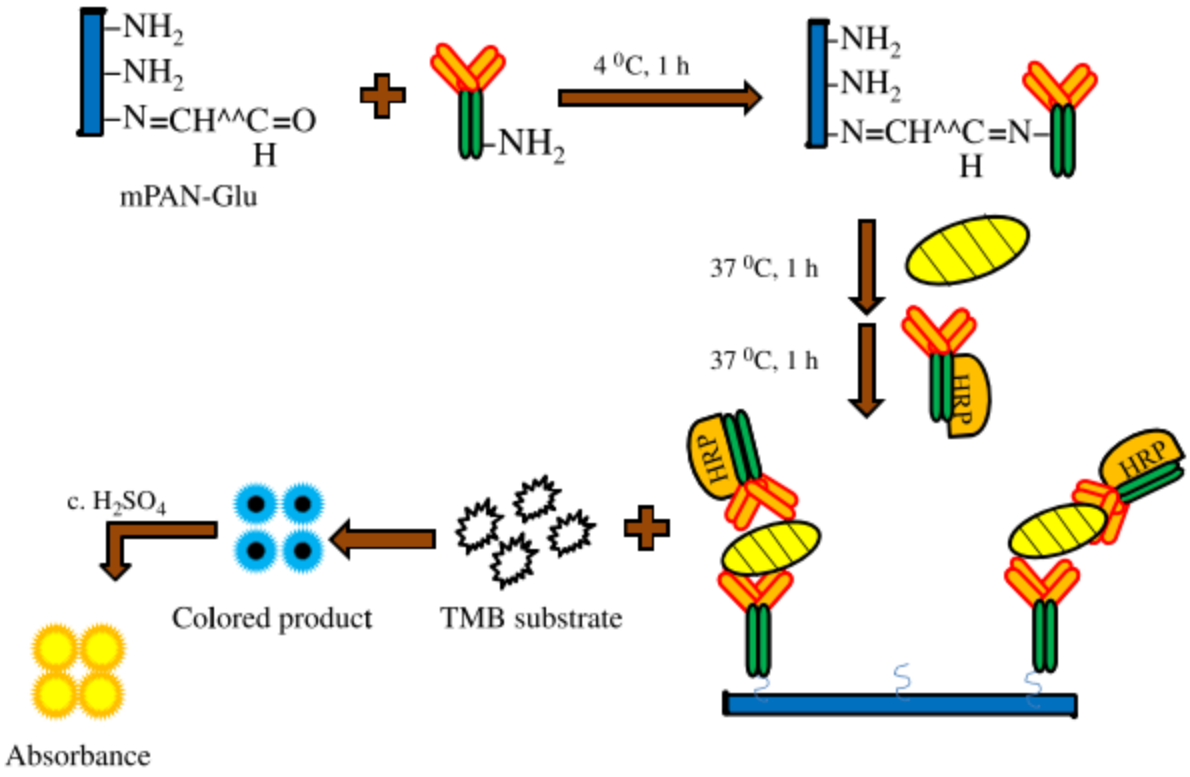

Figure 7. Schematic representation of immunoassay for the detection of *S. typhimurium* bacteria developed on modified PAN fibers

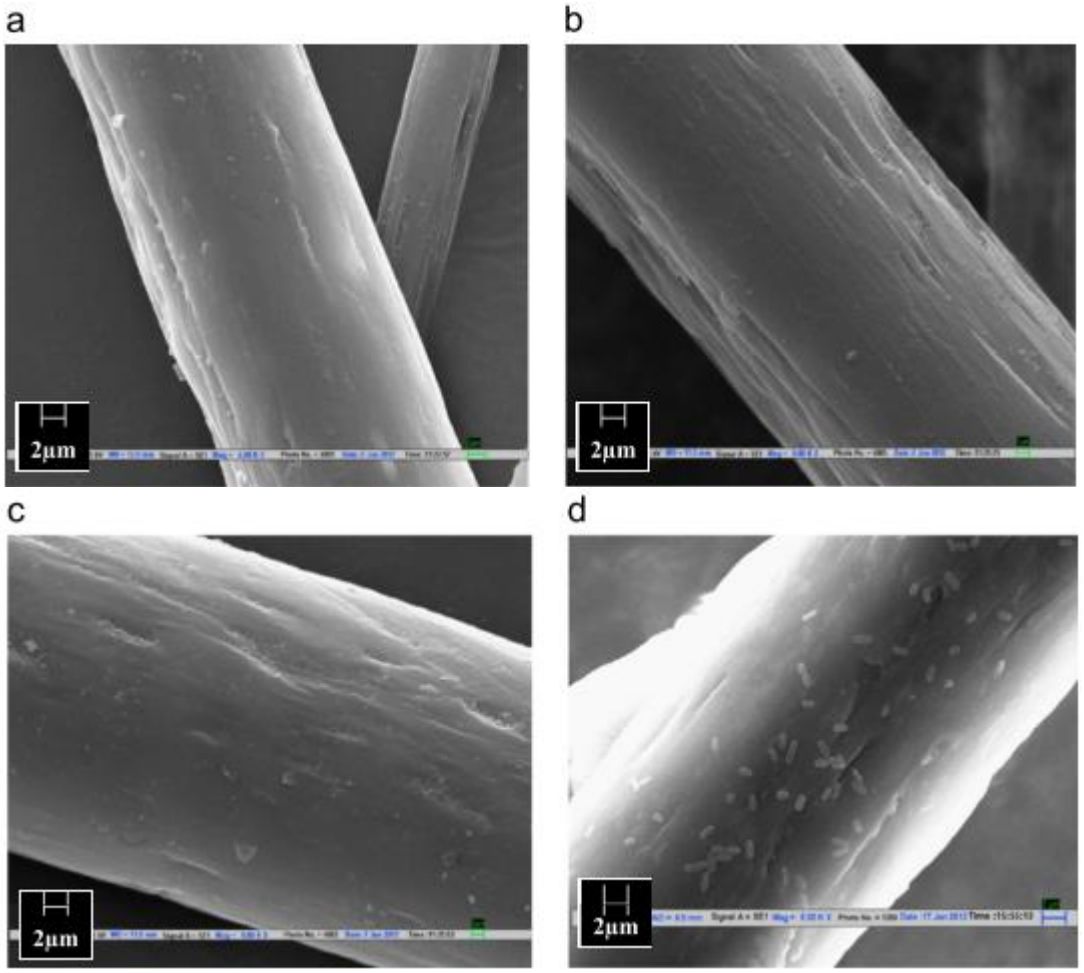

Figure 8. Scanning electron micrographs of (a) virgin, (b) surface aminated, (c) CSA-1-Ab immobilized and (d) *S. typhimurium* captured fibres. (Sruti Chattopadhyay, 2013)

Table 2. ELISAs developed for toxin detection in food

Analyte	ELISA type	Food matrix	LOD
Ochratoxin A	Direct competition	Maize, barley, soy	0.5 ng/g
Listeria monocytogenes	Sandwich (direct, indirect,biotin-amplification)	Low-fat milk	$1x10^3$ cells/ml; $3x10^3$ cells/ml
C. perfringens A enterotoxin	Immunomagnetic Separation (IMS)	Meat	2.5 ng/ml
SEA	Sandwich and competitive	Ham paté, turkey paté, milk, sausage, potato mayonnaise, cheese and lemon cake	0.5 ng/g
Aflatoxin M1	Indirect competitive	Milk, milk based-confectionery	0.5 ng/g
Streptomycin, S-derivate, Gentamycin, neomycin	Different	Milk	ns
SEA	Microsphere-packed capillary/sandwich	Ham, cheese, chicken, bean sprouts	1 ng/g
SHE	Sandwich	Mashed potato (raw milk), sausage, bulk milk	55.5 ng/g
SEA-SED	Indirect double sandwich	Cheese, chicken, salad, milk, cream	1 ng/g
SEB	Distick sandwich	Cheese	0.5-1 ng/g
Aflatoxin M1	Supermagnetic nanoparticles (direct competitive)	Milk	4 ng/ml
C. butulicum BoNT A, B, E, F	Amplified/sandwich	Soft drink, juice, bottled water, vanilla extract, ice cream, honey, milk, legumes, spices, turkey, sausages, beef	2 ng/ml
C. butulicum BoNT B	BoNT/B endopeptidase	Pate, cheese, cod, mince, sausage	10 pg/ml
Fumonisin B1	Indirect competitive	Com	80 ng/g
Nivalenol, T-2, H-2	Competitive	Wheat kemels	30 ng/g T-2

LOD: limit of detection; ns: not specified (Moises, 2009)

Furthermore, Pt-NPs-modified microchannels in a monolithic amorphous carbon electrode are produced by high-temperature treating poly(methyl methacrylate) (PMMA) electrospun onto a Si substrate treated with PAN/chloroplatinate. By adding carboxylic acid groups, plasma treatment makes it easier to graft antiaflatoxin B1 (AFB1) antibody using EDC/NHS at the electrode, where the microchannels function as nanorectors for the interaction of the antibody with the antigen. Better charge transport occurs in the channels as a result of this. AFB1 antigen may be detected by the sensing platform at a limit of detection (LOD) of just 1 pg/mL (Iftikhar et al., 2023).

Surface Plasmon Resonance

SPR has great potential for studying surface-confined affinity interactions without rinsing out excess or unreacted reactants in sample solutions because it is a surface-sensitive optical technique for monitoring biomolecular interactions occurring close to a transducer (gold) surface. By taking advantage of the interfacial refractive index changes associated with any affinity binding interaction, it enables real-time study of the binding interactions between a biomolecule (antibody) immobilized on a transducer surface and its biospecific partner (analyte) in solution without the need to label the biomolecules (Ambrosetti et al., 2022; David et al., 2022; Dhesingh Ravi Shankaran, 2007). Since its introduction in the early 1990s, surface plasmon resonance (SPR) has been widely used in biomaterial characterization studies, drug discovery ligand-fishing kinetics, and the detection of a wide range of chemical and biological materials. Protein binding, association/dissociation kinetics, affinity constants, and other significant aspects were studied through the use of SPR, and these findings contributed to a wider range of application areas, including molecular engineering, food analysis, clinical diagnosis, proteomics, environmental monitoring, bacteriology, virology, cell biology, drug discovery, and warfare detection (Chen et al., 2020; Dhesingh Ravi Shankaran, 2007; X.D. Hoa, 2007).

SPR principle

Surface plasmon resonance is explained as a charge density oscillation that occurs at the interface between two media with oppositely charged dielectric constants. It is related to the evanescent electromagnetic field that is generated on the surface of a thin metal film when excited by an incident beam of light of the proper wavelength at a specific angle. SPR facilitates the detection of only surface-confined molecular interactions taking place on the transducer surface because the evanescent field generated under total internal reflection conditions is strongest at the interface and diminishes exponentially with increasing penetration distance from the interface (Dhesingh Ravi Shankaran, 2007; Saftics et al., 2021). The evanescent waves can resonate with surface plasmons (SP), which are generated by free electrons on the metal film of the sensor surface, at a specific incident light wavelength or angle. The energy of the incident light will be absorbed by SP, causing an arrow dip in the reflected light spectrum.

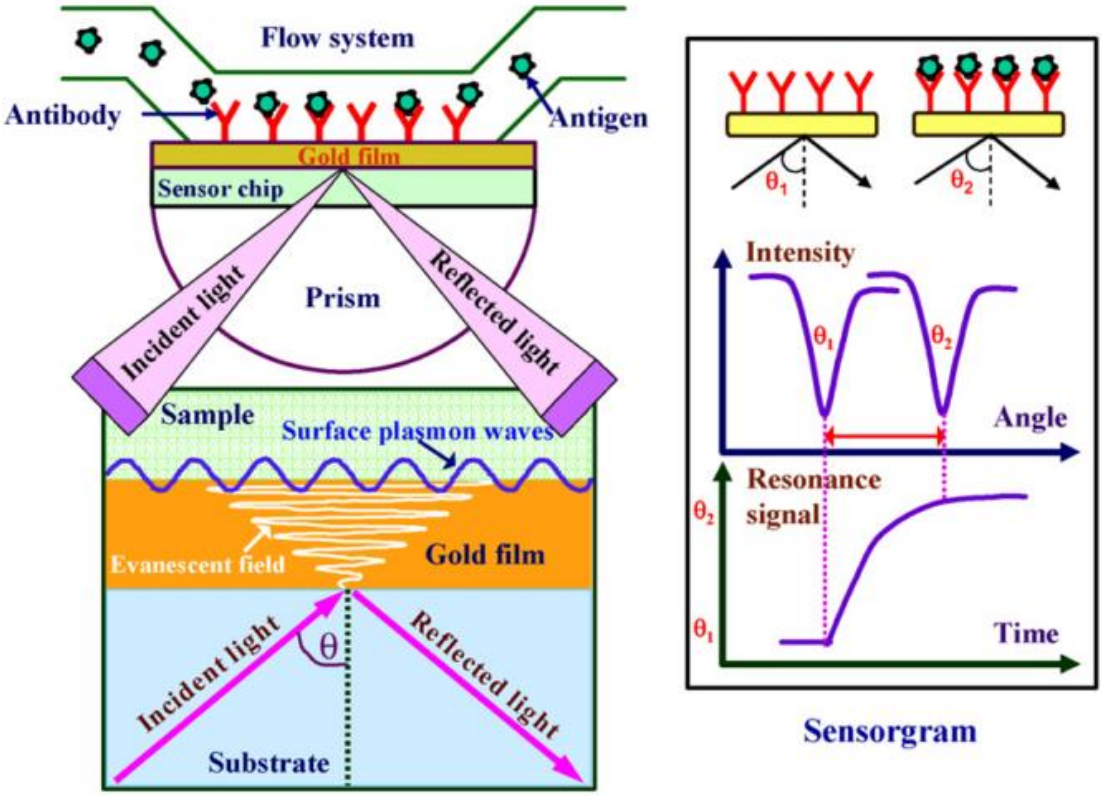

Figure 9. Schematic view of the surface Plasmon resonance immunoassay technique. (Dhesingh Ravi Shankaran, 2007)

The SPR angle is the angle at which the drop is greatest (minimum reflectivity). The species and quantity of biomolecules immobilized on the gold layer have a significant impact on this SPR angle, which is also highly sensitive to the refractive index of the sample contacting the metal surface (Xhoxhi et al., 2015; Zhang et al., 2021). Additionally, (Ying Li, 2012) states that kinetics information regarding the interaction between molecules can also be obtained. Surface plasmons are excited using one of two configuration types: Kretchmann or Otto. For exciting surface plasmons, most SPR instruments employ the Kretchmann configuration operating at attenuated total reflectance (ATR) (Dhesingh Ravi Shankaran, 2007; Ying Li, 2012).

Application to food analysis

In the food industry, biosensors are hoped to detect a wide range of individual analytes, such as veterinary drugs in animal products like dairy, meat, and fish, pesticides or herbicides in agricultural products, food preservatives, nutritional additives, and so forth (Amit et al., 2017). Depending on the type of field application, analytical techniques used in the food industry can be divided into two main groups. Mostly, periodic routine analysis at industrial establishments requires automated analytical systems with high throughput and low cost. However, during pandemic outbreaks, quick, compact field-portable sensor systems are highly valued for monitoring food safety, preventing food adulteration, and enforcing dietary restrictions. In order to detect different analytes related to food, both miniaturized transportable SPR immunosensor systems and traditional laboratory-based systems are being developed (Janik-Karpinska et al., 2022; Naresh & Lee, 2021). Table 3 lists the main SPR immunosensor systems that have been looked into for use in the food industry (Dhesingh Ravi Shankaran, 2007).

Primarily based on the indirect competitive immunoreaction principle, SPR immunosensors were used in the detection of different small-molecular organic compounds associated with food quality. Based on the principle of indirect inhibition immunoassay, an SPR immunosensor of domoic acid, a neuroexcitatory toxin derived from marine diatoms and present in sea products, reported a low detection limit of 0.5 ppb (Dhesingh Ravi Shankaran, 2007; Qiuming Yu, 2005). Nivalenol and deoxynivalenol are toxic fungal metabolites. Tomoyuki Kadota et al. reported using an SPR immunosensor to detect these metabolites. The half maximal inhibitory concentration (IC50) values of the SPR assay were 14.9 $ngmL^{-1}$ for deoxynivalenol and 28.8 $ngmL^{-1}$ for nivalenol, respectively (Tomoyuki Kadota, 2010). Folic acid is a common nutrient in fruit drinks, infant formulas, and sports supplements. SPR immunosensor was reported for the direct detection of folic acid from fortified food samples as low as the admissible concentration levels (Dhesingh Ravi Shankaran, 2007; Malin BostroÈm Caselunghe, 2000). It was discovered that SPR immunosensors, which combine the high sensitivity of SPR technology with the high specificity of immunoassay techniques, were effective in removing, to a minimum of 0.1%, adulteration of milk powder with peanut, soy, or wheat proteins (Dhesingh Ravi Shankaran, 2007; Neethirajan et al., 2018). Using the direct immunoassay method, an SPR immunosensor was shown to be able to detect live microorganisms including *Salmonella paratyphi* and *Escherichia coli* in addition to preservatives, nutrients, and metabolites of fungi and microbes (Byung-Keun Oh, 2004; Dhesingh Ravi Shankaran, 2007; Guliy et al., 2023; John Waswa, 2007; Wang et al., 2022). The SPR sensor system is appealing for a variety of applications in the domains of food quality,

safety, and security due to its quick analytical capabilities, automation, low sample consumption, and high sensitivity to all types of analytes (Dhesingh Ravi Shankaran, 2007). With the sandwich immunoassay, a limit of detection (LOD) of 4×10^4 CFU/mL was discovered for bacterial cells. It was also designed to detect SEB using a sandwich SPR-immunosensor. Using carboxymethyl-dextran attachment, the anti-SEB Abs were covalently linked to the gold-chip surface. SEB was found at 10 ng/mL by the SPR-biosensor test in 8 minutes (Janik-Karpinska et al., 2022).

Electrochemical Immunosensors

Potentiometric, amperometric, and conductimetric electrochemical techniques can all be used for analytical purposes. Nevertheless, due to their high sensitivity, affordability, and potential for instrument miniaturization, amperometric detection systems have been shown to be the most effective method for building immunosensors (Baranwal et al., 2022; Ndunda & Mwanza, 2023). Amperometric detection typically uses a three-electrode system, although in many cases, this is reduced to two electrodes in practice. It is based on the measurement of a current at a fixed (potentiostatic technique) or variable (voltammetric technique) potential. The species of interest are either reduced or oxidized at the working electrode by applying a specific potential between the working and reference electrodes.

This causes an electron transfer, which produces a measurable current directly proportional to the concentration of the electroactive species at the electrode surface over a wide dynamic range (Lazanas & Prodromidis, 2023; Ricci, 2007).

The use of single-use screen-printed electrodes (SPEs), which are inexpensive to fabricate and can be produced in large quantities, has sparked interest in the development of immunosensors (particularly enzyme immunosensors) in recent years. Different inks are printed onto planar ceramic or plastic supports using screen-printing (thick-film) technology (Hang Wei, 2007; Martínez-Periñán et al., 2020; Paimard et al., 2023; Ricci, 2007).

Table 3. SPR immunosensor developed for food-related applications

Analyte	Application	Technique	Determination range/LOD	Fabrication method/features
Damoid acid	Neuroexcitatory toxin from marine diatom, shellfish contamination	SPR, indirect inhibition immunoassay	0.5-150 ppb	Covalent binding of analyte derivative on OEG monolayer
Damoic acid	Neuroexcitatory toxin from marine diatom, seizures and memory loss	SPR, indirect inhibitory immunoassay	2 ppb-3.3 ppm	Thin molecular-imprinted polymer layer
Penicillin and its derivatives	β-Lactum antibiotics, raw and defatted milk samples	SPR, direct inhibition immunoassay	2 ppb	Covalent binding of antibody on CM-dextran layer
Penicillin and its derivatives	β-Lactum antibiotics, milk sample	SPR, indirect inhibition immunoassay	1.5-5 ng/g	Covalent binding of analyte derivative on CM-dextran layer
Folic acid (health supplement)	Fortified foods for children, athretes, milk, infant formulae and cereal samples	SPR, indirect inhibition immunoassay	18 ppb	Covalent binding of analyte derivative on CM-dextran layer
Tylosin residues	Antibiotics against bacterial pathogens in apiculture, honey samples	SPR,indirect inhibition immunoassay	2.5-10 ppb	Covalent binding of tylosine on CM-dextran layer
Deoxynivalenol	Mycotoxin from fusarium fungi	SPR, indirect inhibition immunoassay	2.5-30 ppb	Covalent binding of a conjugate of casein
Non-milk proteins	Microorganisms, foodborne pathogens	SPR, direct immunoassay	200-5000 ppb	Covalent binding of antibodies on CM-dextran layer
Salmonella paratyphi	Microorganisms, foodborne pathogens	SPR, direct immunoassay	10^2-10^7 CFU/ml	Immobilization of antibody over protein G layer
E. coli 0157:H7	Food pathogens	SPR, direct immunoassay	10^4 cells/ml	Immobilization of antibody over protein G layer
Aflatoxin B_1	Lethal fungal metabolites (aspergillus species), maize and peanuts	SPR, indirect inhibition immunoassay	3-98 ppb	Covalent binding of protein conjugate on CM-dextran layer
Aflatoxin B_1	Lethal fungal metabolites (aspergillus species), maize and peanuts	SPR, indirect inhibition immunoassay	0.3-12 ppb	Covalent binding of analyte on CM-dextran layer

(Dhesingh Ravi Shankaran, 2007)

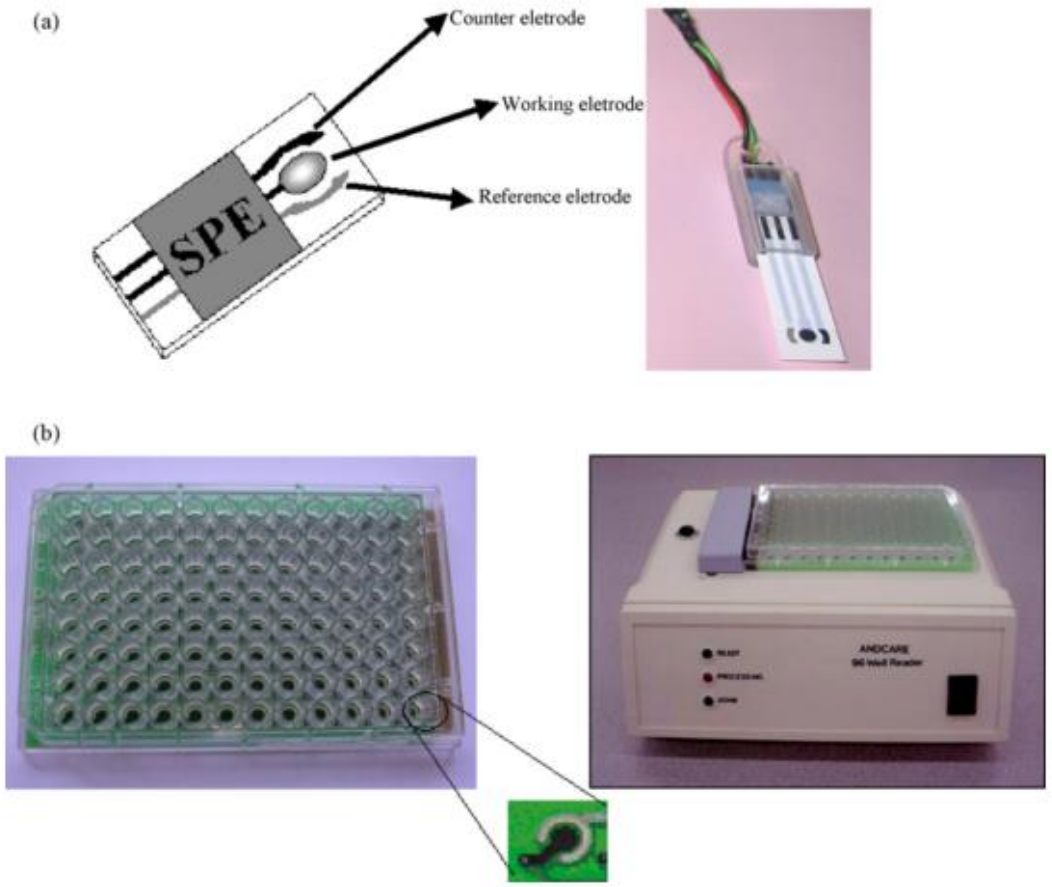

Figure 10. (a) Schematic view of a typical screen-printed electrode (left) and image (right). (b) Multichannel electrochemical immunoassays (MEI) with an instrumentation reader and a close-up of a single well on the left (Ricci, 2007)

Table. 4. Some examples of nanomaterial based biosensors applied in food analysis (Merkoci, 2011).

Biosensor type	Analyte	Sample	Nanomaterial used	Limit of detection
Electrochemical	Pathogens			
	Salmonella spp.	Pork	Au NPs (ca.25 nm)	$1.0x10^2$ CFU/ml
	E. coli 0157:H7	Milk	Au NPs	50 CFU/strip
	E. coli	Surface water	Cu/Au NPs	3 CFU/10 ml
	Salmonella typhi	Phosphate buffer solution	Au NPs (ca. 15 nm)	98.9 CFU/ml
	Pesticides			
	Methyl parathion and chlorpyrifos (insecticides)	0.002M phosphate buffer solution (pH 7.0)	SWCNT	$1x\ 10^{-12}$ M
	Parathion (insecticide)	Buffer solution (pH 2.0-7.0)	ZrO_2 NPs (ca. 22 nm)	3 ng/ml
	Paraoxon (insecticide)	0.05 M phosphate buffer (pH 7.4)	MWCNT	150 nM (S/N= 3)
	Sugars			
	Glucose	50 mM phosphate buffer solution (pH 7.4)	xGnPs (thickness of 10 nm) decorated with Pt an Pd NPs (ca. 1-4 nm diameter)	1 µM (S/N = 3)
	Glucose	0.1 M phosphate buffer solution (pH 7.4)	ZnO:Co Nanoclusters (5 nm)	20 µM (S/N = 3)
	Glucose	Fruit juice	Au NPs	-
	Fructose	0.1 M phosphate buffer solution (pH 5.0)	MWCNT (ca. 5-10 nm diameter)	Ca. 5 $mmoldm^{-3}$
	Fructose	Honey	SWCNT	$1x\ 10^{-6}$ M

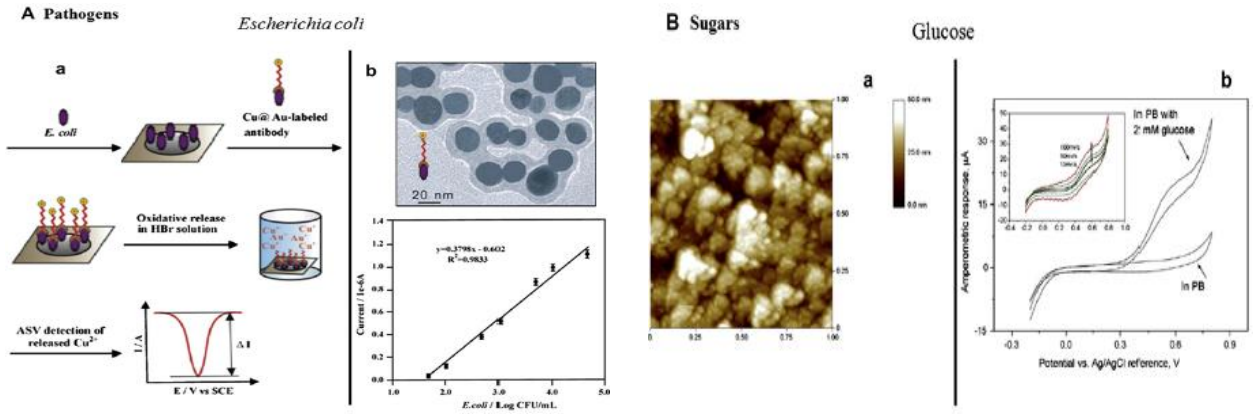

Figure 11.

(A) *Escherichia coli* (*E. coli*) pathogen electrochemical biosensing. (a) The electrochemical immunoassay's general design, which uses Cu@Au NPs as anti-E. coli antibody labels. (b) Cu@Au-labeled antibody TEM image (upper part); (lower part) Plot of calibration used for the anodic stripping voltammetry (ASV) to measure the concentration of *E. coli* and the released Cu2ξ. Reproduced with authorization. (B) Sugar biosensory electrochemical As in glucose. (AFM) image of the thin film electrode made of assembled ZnO:Co nanoclusters. (b) Voltammetric responses of the modified electrode containing glucose oxidase in 0.1 M PBS (pH 7.4) and PBS with 2 mM glucose, using the Nafion-assisted cross-linking technique to create a ZnO:Co nanocluster-assembled thin film. The response to various scan rates is displayed in the inset plot (Merkoci, 2011)

An SPE's scheme is shown in Figure 10a. Because SPEs are so small, only a few microliters of solution are needed for each immunological step, which minimizes the amount of reagent used. The use of such electrode material has become increasingly popular in recent years due to new products that are based on the use of screen printed electrodes. For instance, a disposable multichannel immunochemical sensor printed on a ceramic substrate that consists of an array of eight gold working electrodes and a silver reference electrode was recently developed. Recently, a novel analytical immunosensor array has also been proposed, which combines the high sensitivity of the electrochemical transducer with the simplicity of the spectrophotometric microplate. The used disposable sensor array is a screen-printing device that is made of a modified 96-well plate with an array of 96 screen-printed sensors on the bottom (Figure 10b). It uses intermittent pulse amperometry (IPA) for operation. The multichannel electrochemical immunoassay (MEI), a potentially very helpful tool that offers the rare opportunity of combining the high sensitivity of electrochemical SPE-based immunosensors with the advantageous features of high throughput ELISA procedures, was developed using a 96-well electrochemical plate (Ricci, 2007; Wills et al., 2021).

Applications in food analysis

Antibody-modified nanoparticles are demonstrating improvements in the immunological method for Salmonella spp. detection. For instance, monoclonal antibodies (McAbs) against immobilized Salmonella spp. on Au NPs. The electrochemical impedance spectroscopy (EIS) technique was used to assess how well McAbs interacted with *Salmonella spp.* to effectively create a capacitive immunosensor for detecting *Salmonella spp.* in pork samples. Enterotoxigenic *E. Coli* (ETEC), enteroinvasive *E. Coli* (EIEC), enterohemorrhagic *E. Coli* (EHEC), enteropathogenic *E. Coli* (EPEC), and enteroaggregative *E. Coli* (EAEC) are important microorganisms of interest to be analyzed. Certain strains of this organism have the potential to induce diarrhea, UTIs, inflammations, and peritonitis in patients with compromised immune systems, including the elderly and children, thus, evaluating the quality of food and drinking water is crucial. The microbe *E. Coli* O157:H7 is responsible for the potentially fatal hemolytic-uremic syndrome (HUS) (Merkoci, 2011).

Conclusion

The prevalence of foodborne poisonings remains a significant concern for public health, with more than 200 recognized diseases associated with foodborne pathogens. Bacteria, particularly *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and enteropathogenic *Escherichia coli*, are the primary culprits behind over 90% of recognized pathogen-related food poisonings each year. These bacteria are predominantly found in raw foods. Traditional methods of food safety testing, while sensitive, are time-consuming and labor-intensive. The Hazard Analysis Critical Control Point (HACCP) system is recognized as an effective approach to ensuring food safety, emphasizing prevention at every stage of the food chain. Innovative technologies such as quantitative polymerase chain reaction (PCR) and biosensors offer promising alternatives for rapid and sensitive detection of pathogens and toxins in food samples. Biosensors, in particular, demonstrate high sensitivity and specificity, enabling quick detection of a wide range of analytes in complex samples. Surface plasmon resonance (SPR) immunosensors, based on the principle of indirect competitive immunoreaction, show great potential for detecting various organic compounds and toxins associated with food quality, including neuroexcitatory toxins and fungal metabolites. As the food industry seeks to enhance food safety measures, the development of simple, rapid, and automated analytical systems becomes imperative. These systems should be adaptable for both routine analysis in industrial settings and portable field applications during outbreaks or for on-site monitoring. Technologies such as SPR immunosensors, with their ability to provide quick and sensitive detection across a variety of analytes, offer promising solutions for ensuring the quality and safety of food products.

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