Nanobiosensors, as a Next-Generation Diagnostic Device for Quality & Safety of Food and Dairy Product

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Abstract

Nanotechnology and biotechnology are a sole combination and generate a new advanced discipline: nanobiotechnology. It includes the application of the tools and processes of nanotechnology to study and manipulate biological systems. Nanobiosensor is an advanced, analytical technique designed and developed by nanotechnology and biotechnological applications. They have remarkable benefits with cost-effective, high selectivity, specificity, more rapid, robust, and sensitive quantitative techniques for field analysis capability. These techniques have proved to be the best tools to ensure food and dairy product quality and safety. This chapter focuses on the potential role of nanomaterials (gold, magnetic, carbon nanotube, silver nanoparticles, etc.), which are currently being used in various nanobiosensors to find various chemical adulterants, different toxins, and harmful pathogens. Analysis of food and dairy products for adulterants, such as starch, urea, hydrogen peroxide, neutralizer, detergents, boric acid, melamine, mycotoxins, and bacteria, is performed. Various nanobiosensor used for this purpose are nanowire biosensors, ion channel biosensor, cantilever biosensor, and optical biosensor.

Keywords

Nanobiosensor • Adulterants • Diagnostic device

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7.1 Introduction

Nanotechnology is a new developed discipline that has scientific equipment and modified material that involves changes in chemical and physical properties of any matter at molecular or nano levels. Alternatively, the technique and knowledge of basic biology are used by biotechnologists to change at the level of genetic, molecular, or nano and cellular processes for the development of products and services. Its use ranges in areas from medicine to the agriculture field. Nanotechnology and biotechnology are the sole combination and generate a new advanced discipline: nanobiotechnology. Nanobiotechnology is biotechnology at the nanoscale. It includes the application of the tools and processes of nanotechnology to study and manipulate biological systems (Fakruddin et al. 2012). By using this technique, nano or molecular level instruments can be formed by simulating or using the technique of basic biology to make tools to control and study different properties of living systems at the molecular level. Nanobiotechnology is an innovative science and easier for the life science or many field current issues. This technology has the capability to design and give a shape of our recent concept and simplify the understanding by removing borders between biology, physics, and chemistry. Therefore, new challenging concepts and directions give us better facilities in the field of diagnostics and other disciplines limited by the extensive use of nanobiotechnology with the passage of time.

7.2 Nanotechnology: Impact on Food and Dairy Product Quality and Safety

Recently, there has been increased awareness of consumers for food and dairy product safety and quality. An important requirement is to provide a high-quality and safe dairy product. Mainly conventional methods are used to check quality of food and dairy products, and it is high sensitivity and accuracy but is expensive and requires trained manpower. Analysis requires a rapid, reliable, and cost-effective method.

To ensure food and dairy product quality and safety, analysis of such food and dairy products for adulterant, such as starch, urea, hydrogen peroxide, neutralizer, detergents, boric acid, melamine, and mycotoxins, is very important. Comparison with presence of adulterants is important to follow the minimum limits set by governmental food authorities worldwide. Most analysis tests of these adulterants are analyzed by conventional methods (Vidal et al. 2013). However, new techniques have been develop to analyze these adulterants, i.e., nanobiosensor technique, which is a quick, reliable, sensitive, and cost-effective measureable method for the field level testing ability. By applying these techniques, the nanomaterials used in sensor, i.e., nanobiosensor development is more benefiting in adulterant analysis and developing ultrasensitive devices with nanobiotechnology and its impact.

Recent developments performed in the field of nanotechnology and bionanotechnology permit the design of an innovative class of analytical systems: nanobiosensors. The major advantages of nanobiosensors include affordability, high degree of automation, highly specific, and selective. Due to these characteristics, an increase in the number of devices has been described in the field of food and dairy product for the diagnosis purpose. The medical sector played a crucial and protuberant role in the development of many novel technologies. Many research efforts were performed for the development of biosensors to check the blood glucose level in a diabetic patient. Later, this application of biosensor was used in new ways in other field applications, such as food, dairy product, and environmental control.

7.3 Understanding Sensor, Biosensor, and Nanobiosensor

In developing countries, the availability of medical diagnostic laboratory is very limited. There is requirement to develop instruments or devices that are convenient, accurate, and inexpensive for the fast analysis or diagnosis of human disease, environmental monitoring, and for food and dairy product safety. Recently, the importance of nanobiosensor and other technology has increased using nanobiotechnology to expose agricultural adulterant.

A *sensor* is a device that is able to identify and respond to input, e.g., light, heat, motion, moisture, pressure, or any other from the physical environment. The signal is an output that is converted to a readable display at the sensor location or transmitted electronically over a network for reading or further processing (Arnold and Meyerhoff 1988). *Biosensor* is "an analytical device that includes a biologically active substance with proper physical transducer to produce a computable signal, which is proportional to the concentration of chemical species in any type of sample" (Belkin 2003; Eggins 2002; Wilson and Gifford 2005; Wilson 2005; Dorothee et al. 2008). *Nanobiosensor* is able to detect any biophysical and biochemical signal associated with a particular analyte (molecule). This presented nanobiosensor can be merged into other technologies, such as lab-on-chip, to facilitate molecular diagnostics or microfluidics device for the analysis of food and dairy product adulterant (Yachna et al. 2009).

7.3.1 Basic Principles of a Biosensor

Biosensor development is the multidisciplinary research field. It requires the involvement of various fields, such as biochemistry, biology, microelectronics, physics at the micro and nanoscale, and surface chemistry. In general, a sensor is defined as a device that can produce an electronic signal resulting in external stimulation and allow the quantification of several physical (pressure, temperature, and mass) or chemical (pH and O_2) properties. Biosensors are biological components, such as protein, enzymes, oligonucleotide, cells, and tissue, included and used to generate particular signal towards the objective component.

A schematic representation of the working principle of a biosensor is shown in Fig. 7.1. Specific biosensor is considered by three main aspects: 1) biological

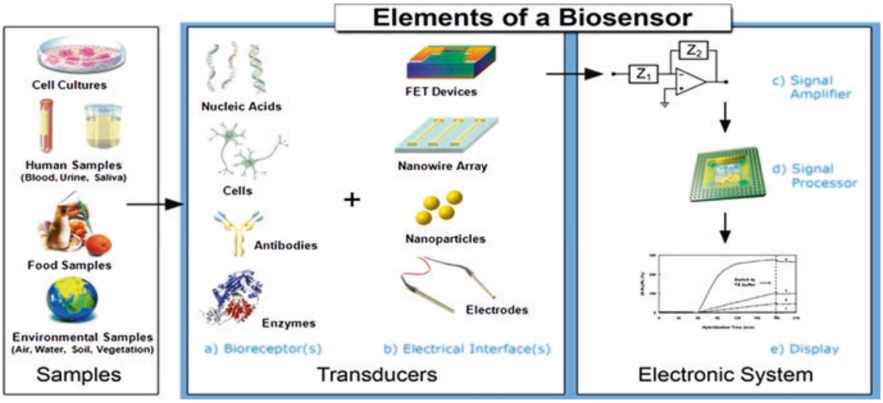

Fig. 7.1 Elements of biosensor includes sample, transducers, and electronic system

recognition element; 2) transducer system; and 3) recognition element with the transducer system (Arnold and Meyerhoff 1988). Examples of nanobiosensors follow.

7.3.1.1 Nanowire Biosensors

One of the nanobiosensors class is nanowire biosensors. The nanowire is made of sensing components, and it is covered by biomolecules, such as deoxyribonucleic acid (DNA), proteins, polypeptides, filamentous bacteriophages, and fibrin.

7.3.1.2 Ion-Channel-Based Sensing

All living cells membrane is formed by protein molecules, and the biological ion channels are hydrophilic sub-nano-sized pores from which biological ion channel fused is formed by protein molecules. Regulation and control of electrical and biochemical activity inside and outside of the cell are monitor through this channel. The development of new generation's nanobisensors control of molecular ion channels is switched by the recognition event.

7.3.1.3 Cantilever Biosensors

Using the flexibility characteristics of cantilever technique can analyze various biomolecules, such as DNA, proteins, vapors, ions, antibiotics, and pathogenic microbes by chemical/biological sensor. The changes in surface of molecules is recognized by static mode techniques, which is more sensitive to molecular interactions, i.e., change in the electronic charge distribution of the substrate's surface atoms and by lateral interactions within the next molecular layer: structural changes and electrostatic forces.

7.3.1.4 Optical Nanosensors

Optical nanosensors are commonly used for the detection of pathogen based on fluorescence and surface plasmon resonance (SPR). This technique is generally based on monitoring the change in the optical signal, which occurs between the functionalized pathogen and nanomaterial. The greatest advantage of this technique is that the sensor can incorporate into the deeper part of cell with negligible disturbance of cell. Nanomaterials, such as gold, magnet, and quantum dots, have strong optical properties that can provide excellent optical labels to improve the sensitivity of optical transducer surface of nanosensor. Optical transducers are mostly attractive for developing the strong device that is easy to use, portable, and cost-effective.

7.4 Nanoparticles in Nanobiosensor

To improve the performance of the existing devices for potential sensing, researchers tend to incorporate nanoparticles used for biosensor construction. Recently, variety of nanoparticles with their *specific* properties, such as small size, high speeds, *lesser* distances for electrons to travel, lower power, and lower voltages. Different types of nanomaterials are utilized, such as super-paramagnetic nanoparticles, bucky balls and carbon tubes, liposomes, nanoshells, dendrimers, quantum dots, and nanorods, to increase the electrochemical signals of biological interaction events that occur at the electrode/electrolyte surface. Functional nanoparticles that attach to biological molecules, such as DNA, peptides, enzyme, and proteins, have been developed for use in nanobiosensors to detect and magnify various signals from biological material (Touhami 2011; Sagadevan and Periasamy 2014). Table 7.1 summarizes the role of various nanomaterials in the detection of contaminants, such as melamine and carbofuran with their minimum sensitivity.

7.4.1 Super-Paramagnetic Nanoparticles

Super paramagnetic nanoparticles bind to a magnetic field but do not hold in magnetism after the field is removed. For the magnetic bioseparation 5–100 nm size in diameter, iron oxide nanoparticles have been used. Many techniques have been used to coat the iron oxide nanoparticles with gold, silica, antibodies of cell-specific antigens, and for separation from the medium. It also is used for drug delivery and gene transfection in membrane transport studies. External magnetic field can accelerate the magnetic nanoparticles towards target tissue along with bioactive molecules or DNA vectors and drugs. It also is useful as magnetic resonance imaging contrast agents (Basa 2007).

7.4.2 Bucky Balls and Carbon Tubes

Bucky balls and carbon tubes are structural class of fullerene. Bucky balls are round in shape, whereas carbon tubes are tubular. The size of a carbon tube is several nanometers in diameter, but the length can be much larger. It can be several millimeters, depending on its use. Due to strength and unique electrical properties of carbon tubes, it has many applications in materials science, drugs, and for vaccines

Table 7.1 Various nanoparticles use to detect food and dairy product adulterants

Nanomaterials	Food and dairy product adulterants	Sensor/probe	Sensitivity	References
Gold nanoparticle	Melamine	Colorimetric probe	0.4 mg/L	Sonawane et al. (2014)
		Standard colorimetric card	1–120 mg/L	Li et al. (2010)
		Surface-enhanced Raman spectroscopy	100–200 μg/L	Zhou et al. (2011)
Water-soluble CdTe quantum dots	Melamine	Fluorescence probe	0.04 mg/L	Mecker et al. (2012)
Single wall carbon nanotube	Melamine	Electrochemical luminescence	1×10^{-13} M	Zhang (2012)
Gold nanoparticle and PB-MWCNTs-CTS	Carbofuren	Electrochemical immunosensor	0.1–1 μg/mL	Liu et al. (2011)
Carbon nanotubes (CNTs)	Food borne Bacterial Pathogens	Electrochemical sensor	1.6 × 104 CFU/mL	Sun et al. (2012)
Magnetic nanoparticles and TiO_2 nanocrystals	*Salmonella*	Optical nanocrystal probes	100 CFU/mL	Jain et al. (2012)
Oligonucleotide-functionalized Au	*Escherichia coli* O157:H7	Piezoelectric biosensor	1.2×10^2 CFU/mL	Joo (2012)
Gold nanoparticles	*Staphylococcal Enterotoxin B*	Chemiluminescence (ECL)	0.01 ng/mL	Chen et al. (2008)
Au NP–PAADs	Brevetoxins	Electrochemical immunosensor	0.03–8 ng/mL	Schofield et al. (2007)
Functionalized-gold nanoparticles	Aflatoxin	Immunoelectrode	10–100 ng dL^{-1}	Yang et al. (2008)
Silver core and gold shell (AgAu)	Aflatoxin B1	Immunodipstick assay	0.1 ng/mL	Tang et al. (2011)
Antigen-modified magnetic nanoparticles and antibody functionalized upconversion nanoparticles (UCNPs)	Aflatoxin B1 (AFB1) and ochratoxin A (OTA)	Immunosensing probes and signal probes	0.01 to 10 ng/mL	Sharma et al. (2010)

(continued)

Table 7.1 (continued)

Nanomaterials	Food and dairy product adulterants	Sensor/probe	Sensitivity	References
Nanostructured zinc oxide	Mycotoxin	Indium–tin–oxide (ITO) glass plate	0.006–0.01 nM/ dm^3	Liao and Li (2010)
Single-walled carbon nanotubes (SWNTs)	Ochratoxin A (OTA)	Fluorescent aptasensor	25–200 nM	Wu et al. (2011)
Fe_3O_4 NPs	*Campylobacter jejuni*	Glassy carbon electrode	1.0×10^3 to 1.0×10^7 CFU/mL	Ansari et al. (2010)

as carriers. Carbon tubes have single and multiple wall structures and other types of tube forms, depending on size, shape, density, and other properties.

7.4.3 Liposomes

The most useful nanoparticle in pharmaceuticals and cosmetic industry is liposomes, because it is composed of lipid and because of its capacity for fusion inside the cells, once their delivery function has been occurred. The first originate nanoparticles used for drug delivery is liposomes, but it cannot release their loaded materials by fusing in aqueous environments and it managed by stabilization using substitute nanoparticles.

7.4.4 Nanoshells

The shape of nanoshells are spherical or round cores because of the specific compound coated into core, i.e., shell or outer coating layer and the thickness of shell is a few nanometers. Application of nanoshells is wide. It is used in biomedicine, pharmaceuticals, cosmetic industry, and diagnosis. The particular wavelengths absorbed by nanoshells depend on the thickness of the shell, which biologically useful. The construction of nanoshells by many materials, such as silica as a core and gold particles attach as a shell. We can replace silica particle with magnetic nanoparticles. Nanoshells such as these have been used to diagnose mycotoxins present in food and dairy products.

7.4.5 Quantum Dots

The only nanoparticles that emit all colours of the rainbow depending on size are quantum dots, also known as nanocrystals. They are semiconductors, nanometer-size particle. Quantum dots are applied in cancer imaging and cell labelling study. It restrains conduction of band electrons and valence band holes in all three special directions. Semiconductor nanocrystals and core-shell nanocrystals are examples of quantum dots.

7.4.6 Nanorods

Nanorods are mostly used in nanomedecines as imaging and contrast agent. The size of nanorods is usually 1–100 nm in length and made up of semiconducting materials, such as gold or inorganic phosphate, small cylinders of silicon, and other materials (Guo et al. 2011).

7.5 Various Methods Used for Synthesis of Nanoparticles

Nowadays, the researchers concentrate on the development of novel, simple, and sensitive techniques to formulate and stabilize nanoparticles. They also work on stable and monodispersed particles formation. By these methods, different metals, metal oxides, sulfides, polymers, core-shell, and composite nanoparticles can be prepared, which are mainly classified into two types: physical methods and chemical methods.

7.5.1 Chemical Methods

7.5.1.1 Chemical Precipitation

Conversion of soluble inorganic materials to soluble impurities was used for removing from the solution by some suitable reagents. It also is removed by flocculated and sedimentation. The removal of precipitate depends on the solubility of the product and is controlled by pH and temperature. Mainly this method is used in industrial wastewater treatment, and recently, researchers used this method for nanoparticle synthesis, such as iron oxide nanoparticles. The major advantage of the chemical precipitation method is that a huge amount of nanoparticles can be synthesized and the control of particle size distribution is limited, because only kinetic factors control the growth of the particles.

7.5.1.2 Vapor-Phase Synthesis

Nanoparticles formulation takes place in gas phase; therefore, this method is known as vapor-phase. In this synthesis technique, atoms and molecules are condensed in gas phase. It is not new; flame reactors are used to produce large quantities of nanoparticles by many multinational companies. Carbon black and titanium dioxide nanoparticles are synthesized by flame reactors.

7.5.1.3 Hydrothermal Synthesis

Hydrothermal synthesis can be defined as a synthesis technique of single crystal, depending upon the solubility of minerals in hot water under high temperature. The growth of the crystal is performed in a stainless steel pressure apparatus, called an autoclave, in which the nutrient media is supplied by dissolving in water. The temperature gradient is kept at the opposite edge of growth chamber so that the hotter end can easily dissolve nutrient media and the cooler end causes seed to take extra growth. The possible merits of hydrothermal technique compared with other typed of crystal growth method include the capacity to generate crystalline stage that is not stable at the melting point. Also in such material, higher vapour pressure near their melting point can be grown by this method. Hydrothermal technique is chiefly suitable for the growth of large good-quality crystal and it maintains good control over their composition.

7.5.1.4 Sonochemical Technique

In sonochemical technique, nanoparticles are synthesized by exposing the aqueous or organic distribution of precursor material using an ultrasonic probe at room temperature. The particle size achieve by this method mainly depends upon the concentration of solution and the time of sonication.

7.5.1.5 Microemulsion Technique

This is a newly innovative technique that can permit the preparation of ultrafine metal particles ranging from 5 to 50 nm in size. The degree of particle nucleation is the function of the filtration rate of micro emulsion droplets. In addition to short introduction on some aspect of microemulsion type and synthesis, we have mainly concentrated on the kinetics of metallic particle synthesis. The barrier of parameters, such as temperature, nature of metal salt, light, and reaction condition, also are studied. This result points out that the nature of stabilizer emulsifier, colloidal stability of microemulsion droplets, and surface activity of additives play crucial role in the particle size and dispersal during preparation of metal particles.

7.5.2 Physical Methods

7.5.2.1 Laser Ablation

In laser ablation, the matter from target surface evaporates with help of high-power laser pulses in such way that the stoichiometry of the material is preserved in the interaction. As a result, supersonic jet of particle (plum) is emitted ordinary to target surface. The plume, parallel to rocket exhaust, spread away from the target with fast forward-directed velocity sharing of the different particles. The condensation of evaporated species will occur on the substrate, which is positioned opposite to the target. The evaporation method occurred in vacuum chamber, either in the presence of some background gas or in vacuum. Oxygen will be the most common background gas in case of oxide films.

7.5.2.2 Sputtering

Sputtering technique is used to cover the thin film of specific material on to the surface of substrate. First, the gaseous plasma is created following acceleration of ions from the plasma into the target material. The target material is eroded by the incoming ions via energy transfer and then emitted in the form of neutral particles. It will be either single atom, bunch of atoms, or molecules. As the neutral particle will emitted, they will run away straight forward until they come in interaction with other particles or nearby surface.

7.5.2.3 Spray Rout Pyrolysis

Considerable size of particles are in nanometer (1–100 nm), which will be of more interest for different applications, extending from electronics via ceramics to catalyst. Due to their distinctive and enhanced property, it is primarily determined by its size, composition, and its structure. Quick, simple, and general method is used for the constant synthesis of nanoparticle with flexible sizes, narrow size distribution, high crystallinity, and better stoichiometry.

7.5.2.4 Inert Gas Condensation

The nanometer-sized particles are synthesized with help of inert gas condensation from metallic iron by evaporation and accumulation in flowing inert gas stream. Inert gas condensation is an expensive but also advanced technique in the field of nanotechnology. This technique is considered as a controlled method. By this technique, the exact shape and size of nanoparticles can be prepared. In this technique, nanoparticles quickly form collides with inert gas in low-pressure environment, and thus controlled and smaller nanoparticles are synthesized (Guo et al. 2011).

7.6 Applications of Nanobiosensor

7.6.1 Nanoparticle-Based Microfluidic Device for Detection of Food and Dairy Product Adulterant

Such a device may be invented using well-established and inexpensive manufacturing techniques, which make it suitable for the detection of adulterant in developing regions. Paper/thread is an alternative material that have the ability to combine with other hydrophilic/hydrophobic material to form contrast and liquid control for fabricating microfluidics system with nanoparticles. Several diagnostic methods can be used in combination with these materials, such as well-established colorimetric detection practice, immunoassay, and many others.

7.6.2 Detection of Allergens in Food and Dairy Products

A novel optical biosensor is developed by combining antibody or aptamer technology with the help of Plasmon Resonance technology, which is used for the detection

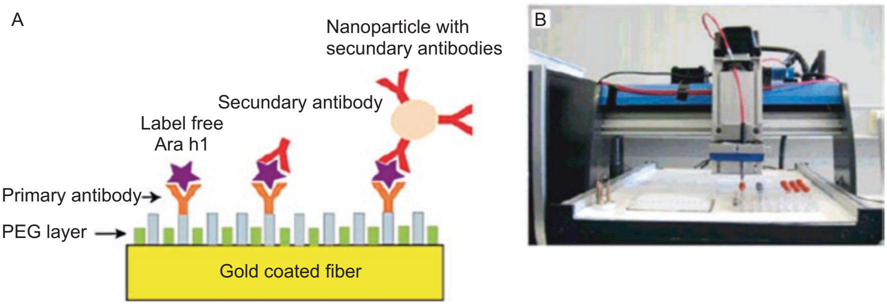

Fig. 7.2 (**a**) shows the working principle of the fiber optic SPR sensor. The bioreceptor molecules are immobilized on the nanoplasmonic sensor module, built around an optical silica fiber, coated with a thin (50 nm) gold layer. When allergens bind to the bioreceptors at the surface, the refraction index changes, which is monitored by an optical detector. Nanoparticles are used for signal enhancement. (**b**) Experimental set-up (Ai et al. 2009)

of allergens in food and dairy products. Biosensor has a couple of merits of optical fiber technology and the use of aptamer as a specific biorecognition element. This biosensor permits rapid, accurate, and label-free screening for the presence of food and dairy product allergens (Fig. 7.2).

7.6.3 Detection of Small Organic Molecules in the Food and Dairy Product

Nanosensors have the capacity to develop quick and accurate technique for industry, and regulatory agencies can detect the existence of food and dairy product adulterant or molecular contaminant in complex food and dairy products. These tests are based on observable color change, which occur to metal nanoparticles media in the presence of analyst, for example, gold nanoparticles (AuPNs) with cyanuric acid, which then selectively bind with melamine. The adulterant is artificially used to inflate the measured the protein content of foods and dairy products. The melamine encourage the accumulation that cause the gold nanoparticles to undergo a reproducible and analyte-dependent color change from red to blue. This can be used to measure accurately melamine concentration in raw milk and infant formula at concentrations as low as 2.5 ppb with naked eyes (Huang et al. 2010).

7.6.4 Detection of Microorganisms in Food and Dairy Products

The capacity to determine that food and dairy products are contaminated by virus, bacteria, or fungi will remain an important research objective. In real food and dairy product system, the most detection methods are needed for the isolation of organism from the nearby environment to confirm that signal-to-noise ratio are sufficiently large to observe. Immunomagnetic separation (IMS) is the technique used to fulfill

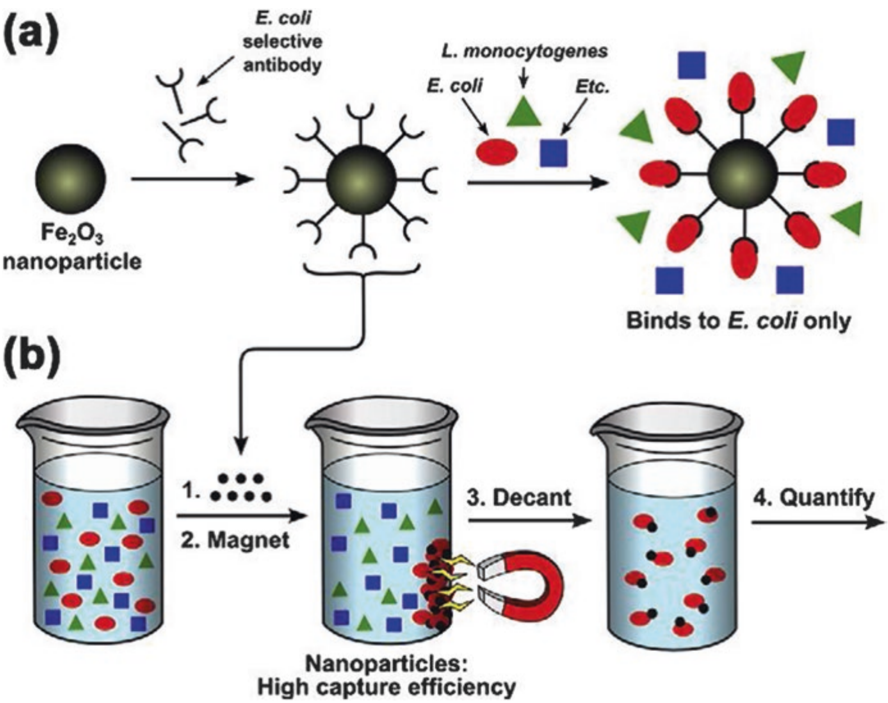

Fig. 7.3 Immune magnetic separation based on bacterial detection technique using magnetic nanoparticles (Ai et al. 2009)

this requirement. In this technique, magnetic particles bind with selective antibodies in mixture with magnet to isolate selectively the target analyte from the mixture of food and dairy product earlier to detection. Nanoscales magnetic particles are widely use in this concern because of their high surface-to-volume ratios, which can help to capture the large analyte efficiencies. Following capturing, the analytes can easily be purified and subjected to standard measurement technique. This technique is illustrated in Fig. 7.3 (Ai et al. 2009).

7.6.5 Detection of Gases in Food and Dairy Products

Oxygen content in food and dairy products is a primary factor that favours the growth of microorganisms. For the purpose of analysis the gaseous elements, the packages of the packed food and dairy products are not early destructed. In processing facilities, packaged food and dairy products are tested randomly during a production run, which is time-consuming, costly, and yet unreliable. A noninvasive method for continuous and easy monitoring of the gas content of a package headspace would provide a means to confirm the quality and safety of the contained food and dairy products long after it has left the production facility. On this view, nanosized TiO_2 or

SnO_2 particle-based photoactivated indicator ink for the detection of in-package oxygen level and redox active dye (methylene blue) has been developed. If a minute amount of oxygen is present, then detector will change colour. Although quantification of the oxygen content in food and dairy product packages might not be possible by this technology, it provides an easy and visual technique to detect modified atmosphere packages (MAPs) with possible compromised seal integrity.

A noninvasive technique for the determination of CO_2 content in MAPs is based on luminescent dyes, which are standardized by fluorophore-encapsulated polymer nanobead. The detecting range of CO_2 sensor is 0.8–100% with resolution of 1% and having 0.6% cross-reactivity with molecular oxygen. Detection of amine gas, which are the indicator of fish and meat spoilage, a hormone that leads to fruit ripening and for the detection of ethylene gas WO_3-SnO_2 nanocomposites, is applied. These all are examples of gas sensing associated with quality and safety of food and dairy products (Joyner and Dhinesh 2015).

7.6.6 Detection of Moisture in Food and Dairy Products

Nanoparticle-based nanosensors also have been developed to detect the presence of moisture content inside a food and dairy product package. Such a nanosensor for moisture detection is based on carbon-coated Cu nanoparticles dispersed in a tenside film. The inter-nanoparticle separation can occur by swelling polymer matrix in a humid environment. These changes in sensor strip can cause reflection or absorption of different color light, which is then easily monitored for rapid and perfect measurement of moisture level in packages without any invasive sampling (Duncan 2011).

7.7 Conclusion

Various nanomaterials integrated to develop various biosensors and nanbiosensors enable the use of biological components to bind and react with a target molecule and transduce generate detectable signals to help rapid detection of food and dairy product adulterants. They can play a crucial role in food and dairy product quality and safety and help to take rapid action when required. Thus, simple visual tests can be created and color changes can easily detected by even unskilled users, making it easier to test for harmful food and dairy product adulterants. From this chapter, it is clear that gold nanoparticles, magnetic nanoparticles, and carbon nanotubes can play a very important role for exposure of adulterants and toxins. Thus, nanobiosensor will be the next-generation diagnostic device for food and dairy product quality and safety. Further research work is required to explore such nanomaterials and new ones that could be used to improve food and dairy product quality and safety.

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