Aptamer-Based Optical Sensors for Food Safety

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Abstract

Food safety is a worldwide problem and ensuring food safety requires strict monitoring of toxins, antibiotics, and bacteria. Thus, sensitive methods of analysis to determine the types and residues of harmful components in food are needed. Point-of-care sensors have been abundantly developed over the last two decades and are proven to be innovative to analyze the contaminants present in food samples both quantitatively and qualitatively. In this book chapter, an overview of aptamer-based optical methods for monitoring food safety is provided. This chapter will focus on optical biosensing techniques such as UV-visible spectroscopy, fluorescence spectroscopy, surface-enhanced Raman spectroscopy, and photonic crystals. The principle, mechanism, advantages, and limitations of each technique are described with an ample number of examples.

Keywords

Food safety · SERS · Toxins · Pesticides · Antibiotics · Biosensors

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6.1 Introduction

Food safety is extremely important for better nutrition, good health, and good quality of life. There is a general propensity to link food and health, to boost well-being and prevent diseases. Any spoilage, deterioration, contamination, pathogenic infestation, or adulteration of food not just leads to degrading its nutritional value, but also consuming these contaminated foods steers foodborne diseases. Foodborne illnesses are a global health problem that contributes to social and economic burden of many countries. According to the World Health Organization (WHO), there are more than 200 food-related diseases that are caused by ingesting contaminated or spoiled food. Approximately 600 million people get sick by contaminated food, of which 4.2 lakhs die every year. Children under 5 years of age are worse affected with 40% of all illnesses and 1.25 lakh deaths every year (World Health Organization 2022). Besides this, foodborne diseases account for economic losses worth billions of dollars around the world.

In the last 30 years, there is an enormous growth in the food sector to fulfill the need of big population and to adapt to changes in lifestyles. Increasing consumption of ready-to-eat food items leads to many socioeconomic and health-related impacts. Many microbiological and chemical changes occur throughout the food processing, distribution, and storage that affects the shelf life and quality of the food, which in turn negatively affects consumer health. Escalating regulatory requirements to control the presence of unwanted or harmful molecules in food leads to increasing food safety concerns. Scientific groups working in food science are constantly required to provide the sufficient answers to the users, whose awareness and concerns for food safety are accelerating (Arduini et al. 2016). Existing methods to assess food safety are chromatographic techniques like gas chromatography, thin-layer chromatography, or high-performance liquid chromatography and advanced techniques such as quantitative real-time polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA). These techniques are costly, time-consuming, laborious, and complex and need heavy instruments and experienced personnel. All these limitations make them difficult to apply in rural areas or resource-limited places where the foodborne diseases are prevalent (Choi et al. 2019). To overcome these problems, biosensors can be a solution (Akhtar et al. 2018, Chandra et al. 2010, and Chandra and Prakash 2020). Over the last two decades, biosensors have emerged as a crucial tool for the analysis of food, and researchers are working on many analytical strategies and technologies for the easy, fast, reliable, sensitive, and economic detection of contaminants, toxins, pesticides, carcinogens, and foodborne pathogens.

Most common biorecognition elements for the development of biosensors are antibodies, enzymes, molecularly imprinted polymers (MIP), and aptamers. Morales and Halpern (2018) have very well explained different types of biorecognition molecules that have been used for the development of biosensors and how to select the biorecognition element for a biosensor. Aptamers are short single-stranded oligonucleotides that are identified by an iterative process called SELEX (selective evolution of ligand by exponential enrichment). They are known as chemical

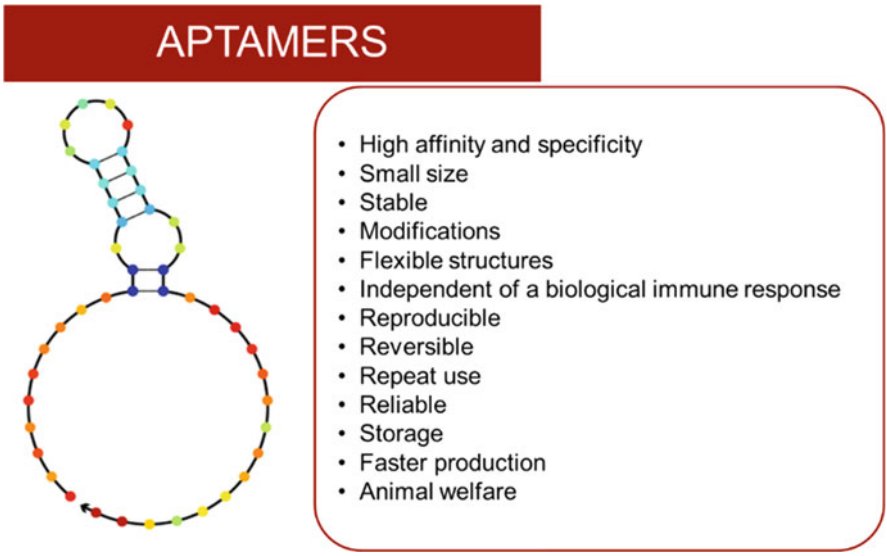

Fig. 6.1 Advantages of aptamers

antibodies, but are easier to produce without any batch difference. Chemical modifications of aptamers are also relatively easy, and therefore, various types of distinct tags can be used for sensing applications (Groff et al. 2015; Kalyani et al. 2021a). In addition to this, they can be extended at both 3′ and 5′ ends for tagging and binding or to incorporate enzymatic activity (Setlem et al. 2020). Aptamers are being used in many biosensors as they have many advantages such as high affinity, small size, no batch variation, easy chemical modifications, and faster production (Fig. 6.1). They have been exploited in conjugation with various detection techniques, viz., fluorescence, SERS, electrochemical, lateral flow assays, and quartz crystal microbalance (QCM), to fabricate biosensors (Dhiman et al. 2017; Arroyo-Currás et al. 2020; Kalyani et al. 2021a; Bayramoglu et al. 2022).

Over the last two decades, there has been exponential growth in the development of optical biosensors as they provide various advantages over other detection techniques. They have been applied for simple, cheap, rapid, and label-free sensing of several chemical and biological molecules in real time. Optical biosensors exploit the interaction of optical field with a biorecognition element that could be an antibody, enzyme, receptor, MIP, or aptamer. They were implemented for clinical diagnosis, food industries, environmental sensing, and medicine. The most commonly used aptamer-based optical methods for food safety applications are colorimetric assays, fluorescence spectroscopy, surface plasmon resonance (SPR), surface-enhanced Raman spectroscopy (SERS), and photonic crystals (Chen and Wang 2020; Asghari et al. 2021; Kaur et al. 2022). The principle, mechanism, advantages, and limitations of each of these techniques are explained with examples in further sections.

6.2 Colorimetric Sensors

It is one of the most widely employed biosensing techniques where target detection can be achieved via observing a change in the color of reaction mixture. The color change is visible to the naked eyes and can be easily quantified using a spectrophotometer (Kalyani et al. 2021b). Aptamer-based colorimetric biosensors employ target-specific aptamers, selected often using SELEX method, and involve conjugation of these aptamers with various nanostructures or enzymes for producing color changes visible to naked eyes. Gold nanoparticles (AuNPs) are most commonly used nanostructures in conjugation with target-specific aptamers where binding of target and aptamer results in salt-induced aggregation of AuNPs and color change. In a study, a highly specific ssDNA aptamer for oxytetracycline in conjugation with gold nanoparticles has been designed where binding of aptamer and target causes release of AuNPs followed by salt-induced aggregation, thereby inducing color change from red to purple (Kim et al. 2010b). Similarly, AuNP-based colorimetric sensors have been devised for detection of mercuric ions (Li et al. 2009), ochratoxin A (Yang et al. 2022), and bisphenol A (Zhang et al. 2016).

In addition to using AuNPs, other novel mechanisms have also been employed for colorimetric assays. In a study a structure-switchable aptasensor has been devised for the detection of aflatoxin B1. Here an aflatoxin B1-specific aptamer and two split halves of DNAzymes having complementary sequence are made to hybridize, resulting in the formation of functional G-quadruplex structure capable of carrying out color change reactions (Fig. 6.2). In the presence of aflatoxin B1, aptamer-DNAzyme complex undergoes structural changes causing release of halves of DNAzymes rendering it incapable of catalyzing color change reaction, thus causing decrease in colorimetric signal (Seok et al. 2015). In the absence of target, the peroxidase-mimicking split DNAzyme is in right configuration and converts light green ABTS to dark green-colored radical anion. When target is present, the DNAzyme remains in split form and no reaction occurs. Table 6.1 includes the list of the colorimetric sensors developed for food safety.

6.3 Fluorescence Sensors

Another type of widely used optical sensor is fluorescence biosensor and used to quantify a number of pollutants and contaminants like heavy metals, antibiotics, food allergens, and toxins. A typical fluorescence-based aptasensor involves the use of a fluorophore and a quencher. Fluorescence-based aptasensors are divided into two groups: (1) "turn-on" fluorescence, i.e., increase in fluorescence can be observed when target molecule binds with aptamer, and (2) "turn-off" fluorescence, where binding leads to decrease in fluorescence (Nsibande and Forbes 2016; Kiruba Daniel et al. 2019; Kalyani et al. 2020). In turn-on fluorescence biosensors, initially fluorophore compound is positioned very close to quencher, and in the presence of target molecule, aptamer-target binding leads to change in its conformation, thus separating the fluorophore from quencher, and increase in fluorescence can be

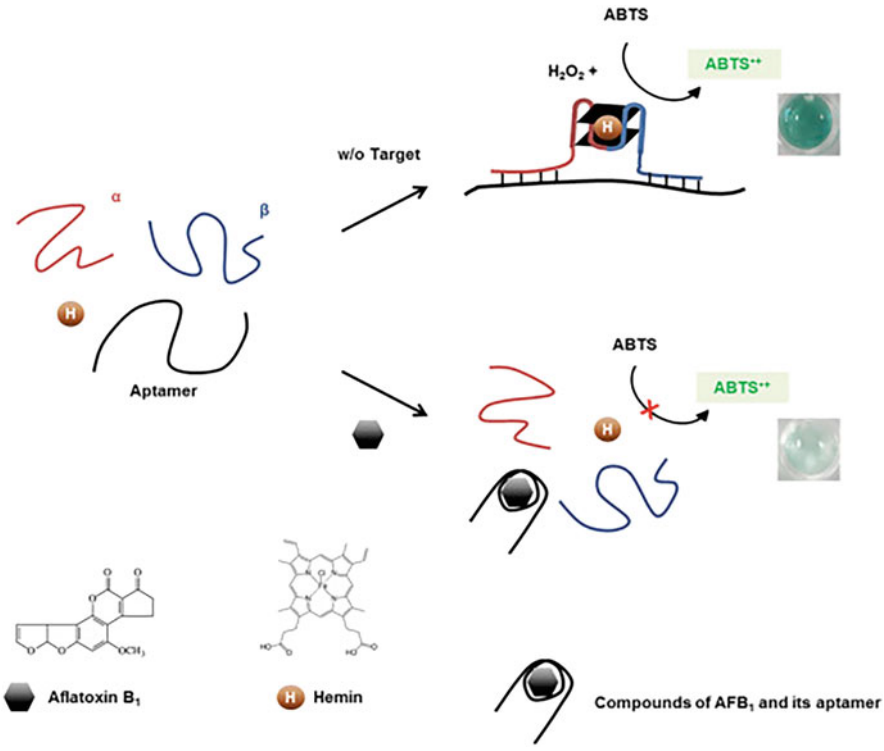

Fig. 6.2 Colorimetric detection of aflatoxin B1 using structure-switching aptasensor. Reused with permission from Seok et al. (2015). Elsevier © 2015

observed. For instance, graphene oxide (GO) sheets have been used to quench the fluorescence of quantum dots in the absence of target molecule (Lu et al. 2015). For the detection of lead (II), the aptamer-QD conjugates are bound to GO sheets, and the resulting energy transfer between QDs and GO leads to quenching. In the presence of lead ions, aptamer-Pb^{2+} binding causes conformational change in aptamer causing the release of aptamer-QD-Pb^{2+} complex from GO sheets, and restoration of fluorescence can be observed (Li et al. 2013). Similarly, using turn-on fluorescence-based technique, CdTe quantum dots-aptamer-GO complex was employed for the detection of aflatoxin B1 (AFB1).

In turn-off sensors, the presence of target molecule brings fluorophore and quencher in close proximity, thus reducing the fluorescence signal, which was otherwise powerful in the absence of target (Akki and Werth 2018). There are other mechanisms which are based on photoinduced electron transfer (PIET) mechanism between fluorescent molecules and guanine residues. For instance, for the rapid detection of ochratoxin A (OTA), carboxyfluorescein (FAM)-labeled aptamer, when hybridized to its complementary strand containing guanine residue at its 3″ end, results in the quenching of fluorescence (Fig. 6.3). In the absence of

Table 6.1 Aptamer-based colorimetric sensors utilized for food safety

Target	Detection mechanism	Linear dynamic range (LDR)	Limit of detection (LOD)	References
Staphylococcus aureus	Utilizes aptamer and dsDNA-SYBR green 1 (SG1) complex	10^2 to 10^7 CFU/mL	81 CFU/mL	Yu et al. (2020)
Vibrio parahaemolyticus	Enzyme-based detection system employing magnetic nanoparticles, gold nanoparticles, and specific aptamers	10 to 10^6 CFU/mL	10 CFU/mL	Wu et al. (2015)
Salmonella enteritidis	Aptamer-based sandwich-type capillary detection platform	10^3 to 10^6 CFU/mL	10^3 CFU/mL	Bayraç et al. (2017)
Salmonella enterica serovar Typhimurium	Aptamer conjugated with gold nanoparticles	10 to 10^6 CFU/mL	10^2 CFU/mL	Sudha et al. (2016)
Aflatoxin B1	Utilizes assembly of aptamer and peroxidase mimicking DNAzyme split probes	0 to 1 ng/mL	0.1 ng/mL	Seok et al. (2015)
Ochratoxin A (OTA)	OTA binding induces conformational changes on OTA aptamer and salt-induced aggregation of AuNPs	20 to 625 nM	20 nM	Yang et al. (2011)
	Aptamer-cross-linked hydrogel which upon interaction with OTA causes hydrogel disruption and AuNPs aggregation	0 to 100 nM	1.25 nM	Liu et al. (2015)
	Gold-conjugated OTC aptamer	25 nM to 1 μM	25 nM	Kim et al. (2010b)
	Lateral flow-based aptasensor employing AuNPs-aptamer conjugates	5 to 50,000 ng/mL	0.254 ± 1.62 ng/mL	Birader et al. (2021)
Cd^{2+}	T- and G-rich DNA aptamer conjugated with gold nanoparticles	10 nM to 4 μM	4.6 nM	Wu et al. (2014)
Bisphenol A	Detection based on interaction among aptamer and bisphenol A followed by AuNPs aggregation	1.50 nM–500 nM	1.50 nM	Zhang et al. (2016)
	Aptamer-bisphenol A binding induced AuNPs aggregation	35 to 140 ng/mL	0.11 ng/mL	Xu et al. (2015)

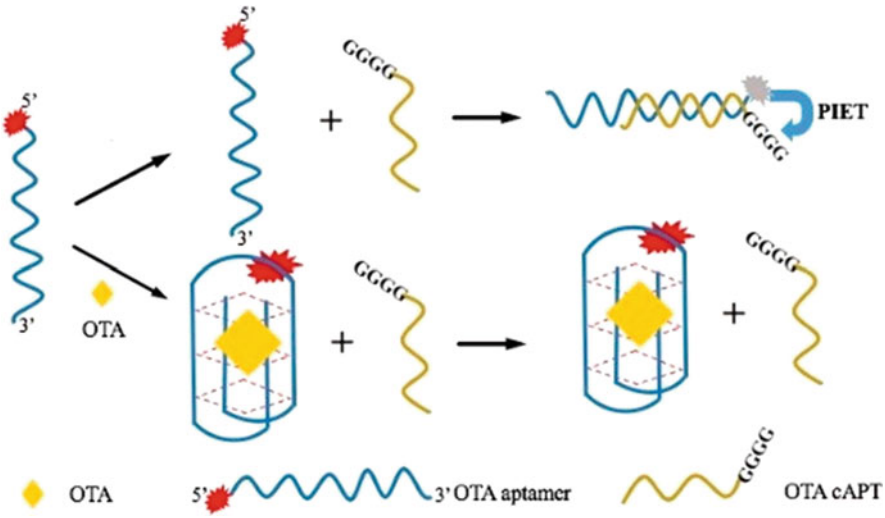

Fig. 6.3 Fluorescence-based sensor for the detection of ochratoxin A. (Zhao et al. 2019)

ochratoxin A, the aptamer is bound to complementary strand that leads to fluorescence quenching. In the presence of target, the complementary strand leaves the aptamer resulting in fluorescence. OTA presence causes the binding of OTA with FAM-labeled aptamer, releasing complementary strand and restoration of fluorescence (Zhao et al. 2019). Table 6.2 highlights some of the aptamer based fluorescensce sensors developed for food safety.

6.4 Surface Plasmon Resonance-Based Sensors

Surface plasmon resonance (SPR)-based sensors rely on the strong oscillation of electromagnetic field at the interface of dielectric medium and nanometal film via p-polarized incident light. At specific incident angle and wavelength of light, this results in a dark band profile of light reflectivity (Prabowo et al. 2018). In SPR, the recognition molecule can be immobilized on chip to capture the target. Various nanostructures have been studied with different SPR properties (Mahato et al. 2018). It provides real-time signals of recognition molecule and target interactions by closely monitoring the change in refractive index. SPR sensors are very sensitive to the external solution refractive index, and the resonant angle and wavelength alter the external solution refractive index. SPR offer advantages of label-free and real-time detection of molecules (Wang et al. 2018). As they are highly sensitive and label-free, they have been extensively used for biological sensing. SPR biosensors have been shown to detect various biomolecules such as virus, proteins, bacteria, and small molecules (Chinowsky et al. 2007; Wang and Zhao 2018; Akgönüllü et al. 2020).

Table 6.2 Aptamer-based fluorescence sensors for food safety

Target	Detection mechanism	LDR	LOD	References
Tropomyosin (food allergen in shellfish)	Aptamer-magnetic nanoparticles complex-based detection	0.4–5 μg/mL	77 ng/mL	Zhang et al. (2018)
Aflatoxin B1	Aptamers are linked to CdTe quantum dots, the fluorescence of which is quenched by graphene oxide	In PBS: 3.2 nM–320 μM In peanut oil: 1.6 nM–160 μM	PBS: 1 nM Peanut oil: 1.4 nM	Lu et al. (2015)
Aflatoxin M1	FAM-labeled aptamer and tetramethylrhodamine (quencher)-modified complementary DNA to aptamer	1–100 ng/mL	0.5 ng/mL	Qiao et al. (2021)
	Carboxyfluorescein-labeled aptamer conjugated to graphene oxide to quench the fluorescence and protect aptamer from DNase I cleavage	0.2–10 μg/kg	0.05 μg/kg	Guo et al. (2019)
Zearalenone (ZEN)	Based on upconverting nanoparticles	0.05–100 μg/L	0.126 μg/kg for corn 0.007 μg/L for beer	Wu et al. (2017)
Ochratoxin A (OTA)	FAM-labeled quencher-free aptamer (F1 and F2) utilizing quenching abilities of guanine for FAM	F1: 0.69–8.0 nmol/L F2: 0.36–4.0 nmol/L	0.69 nmol/L 0.36 nmol/L	Yang et al. (2022)
	RNase H-assisted fluorescence aptasensor	0.4–20 ng/mL	0.08 ng/mL	Wu et al. (2019)
	Quencher-free method based on photoinduced electron transfer between guanine and fluorophore	3 nM–300 nM	1.3 nM	Zhao et al. (2019)
Kanamycin	Reduced graphene oxide-based fluorescent aptasensor	1 pM–20 pM	1 pM	Ha et al. (2017)
Fipronil	FAM-labeled aptamer and tetramethylrhodamine (quencher)-labeled cDNA	25–300 ppb	53.8 ppb	Kim et al. (2020)
Cd^{2+}	Label-free fluorescent aptasensors utilizing PicoGreen as dsDNA-specific dye	0.10–100 μg/mL	0.038 ng/mL	Luan et al. (2016)
Hg^{2+}	Aptamer-templated ZnO quantum dots	0.1–10,000 ppb	0.1 ppb	Kiruba Daniel et al. (2019)

(continued)

Table 6.2 (continued)

Target	Detection mechanism	LDR	LOD	References
Neomycin	DNA	0.003 to 0.72 µg/mL	1.55 ng/mL	Caglayan (2020)
Aflatoxin B1	DNA-chitosan using an N-terminal histidine	0.19 to 200 ng/mL	0.19 ng/mL	Wu et al. (2018)
Aflatoxin B1	Streptavidin attached to CM5 sensor and afterward coated with biotinylated aptamer	0.4 to 200 nM	0.4 nM	Sun et al. (2017)
Tetracycline	DNA tetrahedron-immobilized gold surface	0.01 to 1000 µg/kg	0.0069 µg/kg	Wang et al. (2018)
Kanamycin	CVD-graphene- and rGO-coated gold surface	5.88 to 100 µM	1.79 µM	Écija-Arenas et al. (2021)
OTA	Aptamer nanoparticles Gold nanorods	3.8 to 9 ng/mL NM	3 ng/mL <1 nM	Wei et al. (2018), Rehmat et al. (2019), and Park et al. (2014)

Wang et al. have developed an SPR-based tetrahedron-assisted aptasensor to detect tetracycline (Wang et al. 2018). To reduce steric hindrance between aptamer molecules and improve the accessibility of the aptamer to tetracycline, DNA tetrahedron was used (Fig. 6.4). Using this strategy, aptamer can be oriented in both directions: lateral and vertical, with precisely 6 nm distance. The pyramid structure of the tetrahedron can act as spacer and provide enough space for aptamer to fold properly. It is reported that there was tenfold improvement when DNA tetrahedron is used. In the absence of tetrahedron, the LOD was 0.0183 µg/kg, whereas when aptamer was properly oriented with the help of DNA tetrahedron, the LOD was 0.0069 µg/kg. The specificity of the sensor was tested with close analogues of tetracycline, viz., oxytetracycline and chlortetracycline. For real sample, different types of honey samples were spiked with tetracycline and recovery of 86–114% was observed.

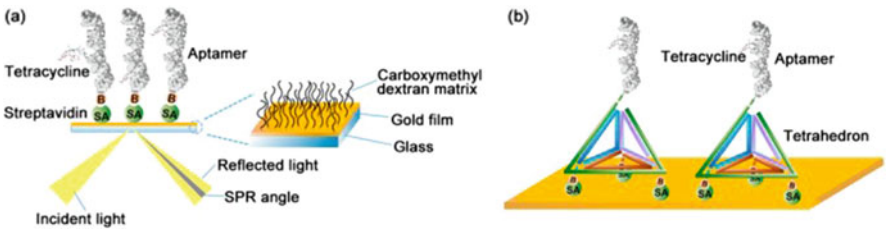

Fig. 6.4 (**a**) SPR aptasensor scheme for detection of tetracycline, (**b**) DNA tetrahedron-assisted oriented immobilization of aptamer

A novel triple-amplification SPR electro-chemiluminescence (ECL) approach was developed by Miao et al. for detecting chloramphenicol (Miao et al. 2016). It is based on horseradish peroxidase enzyme-linked polymer (EV) and single-stranded DNA-binding protein (SSB) attached to gold nanoparticles (EV-Au-SSB) as nanotracer and exonuclease-assisted target recycling. Au nanoparticles in the EV-Au-SSB system efficiently enhance the intensity of ECL of nanocrystals by −1.35 V using SPR technique.

6.5 Surface-Enhanced Raman Scattering (SERS) Sensors

In recent years, SERS has been used for its unique molecular sensitivity and spectral resolution [18] in biomolecular analysis (Lee et al. 2015; Hanif et al. 2017), food industry (Deneva et al. 2019; Muhammad et al. 2020), antibiotics response (Moritz et al. 2010; Kumar et al. 2020), and detection of water pollutants. SERS is an enhancement in the Raman signal from molecules adsorbed on the surface of the SERS substrate. However, this phenomenon can be explained by two general mechanism that are accepted but have distinct factors: electromagnetic enhancement mechanism and chemical enhancement mechanism (Stiles et al. 2008; Ding et al. 2017). The mechanism of electromagnetic factor is mainly based on electric field enhancement caused by LSPR (localized surface plasmon resonance), while chemical enhancement mechanism is based on the charge transfer state between the SERS substrate and molecule absorbed on the nanoparticles (chemisorbed molecules) (Stiles et al. 2008). Therefore, the production of such substrates or platforms is very important, and it's still a huge task to improve the specificity and sensitivity of Raman signals in complex environments like biological samples.

The specificity and sensitivity factor of aptamer-based SERS assays is due to the specific interaction of sample target molecules with the aptamer. There are generally two types of aptamer-based SERS tests: with Raman-labeled molecule (label-based) and without Raman-labeled molecule (label-free). In the label-free strategy, the aptamer can interact with the target molecule and induce conformal changes that alter the aptamer's Raman signal. On the other hand, in Raman labeling method, externally sensitive Raman molecules attached to the detected target help with the measurement. In both the cases, specificity is a main key characteristic of aptamer-based detection, which depends on the target molecule interaction and the capturing DNA sequences of aptamer, and sensitivity depends on the Raman signal generated by various mechanisms of amplification, including the SERS effect, which can be significantly improved.

Since the first application of aptamers in SERS (Barhoumi et al. 2008a, 2008b; Kim et al. 2010a), many SERS biosensors based on diversified aptamers have been developed. It is expected for these sensors to be sensitive and targeted to facilitate quick diagnosis in various fields and applications. Wang et al. review the detection of various substances using aptamer-based SERS sensors (Wang et al. 2019). Similarly, the free use of aptamer labels in Raman spectroscopy is also discussed by Scatena et al. (2019). Table 6.3 lists the SERS based sensors developed for food safety.

Table 6.3 SERS-based sensors for food safety

Target	Detection mechanism	LDR	LOD	References
Acetamiprid	AgNPs@Si-CS as substrate	1 pM to 1 μM	0.30 pM	Shi et al. (2020)
Acetamiprid	AuNP-MMBN aptamer as Raman probe	25 to 250 nM	6.8 nM	Sun et al. (2019b)
Malathion	AgNPs@Si and Ag nanosol substrate	0.5 to 10 μM	1.8 nM	Nie et al. (2018)
Aflatoxin B1	Aptamer-conjugated magnetic-bead and gold nanotriangles	0.001 to 10 ng/mL	0.54 pM	Li et al. (2017)
Oxytetracycline	AuNP tetramer-based aptasensor	0.01 to 250 ng/mL	0.003 g/mL	Wu (2019)
Kanamycin	Au@ag CS NPs	10 μg/mL to 100 ng/mL	0.90 pg/mL	Jiang et al. (2019)
Pb^{2+} Hg^{2+}	Aptamer regulated the production of AuNP Based on the formation of $THg^{2+} - T$ pairs for detection of Hg^{2+}	0.006 to 0.46 mM 10 nM to 1 mM	0.0032 mM 10 nM	Wang et al. (2019) and Lu et al. (2018)

An interesting strategy for using SERS-aptamer-based sensors is to combine them with catalytic reactions (Li et al. 2018a; Sun et al. 2019a; Yang et al. 2020). The fundamental idea is to replace the detection target molecule, which can be more easily detected or enhanced by a catalytic reaction. When encountering biological enzymes, the combination of catalytic amplification and SERS becomes especially important. Basically, the reaction product is enhanced by the introduction of the molecule or the catalytic enhancement of the catalytic activity of the process. The design of target-specific enzymatic reactions is very useful for the development of aptamer-SERS biosensors, especially when enzymes start acting on DNA/RNA.

Fang et al. reported a dual mode of aptamer-attached and enzyme-assisted SERS module for the detection of chloramphenicol (CAP) antibiotic trace at very low section limit of 15 fM (Fang et al. 2019). In this study, a special aptamer was designed against CAP (anti-CAP) which can recognize the alteration in the conformation of the analyte, which initiates the de-hybridization of the target aptamer's DNA (Fig. 6.5a). The addition of a DNA probe labeled with the reporter gene and the exonuclease (III) increases the SERS signal when hybridized with the captured DNA due to the creation of high number of surplus DNA molecules during the amplification loop.

In addition, the catalytic reaction approach can facilitate the generation of SERS-active nanoparticles that can enhance the SERS signal. Li et al. presented redox-GO reactions for the synthesis of enhanced SERS nanoparticles (Fig. 6.5b) that can

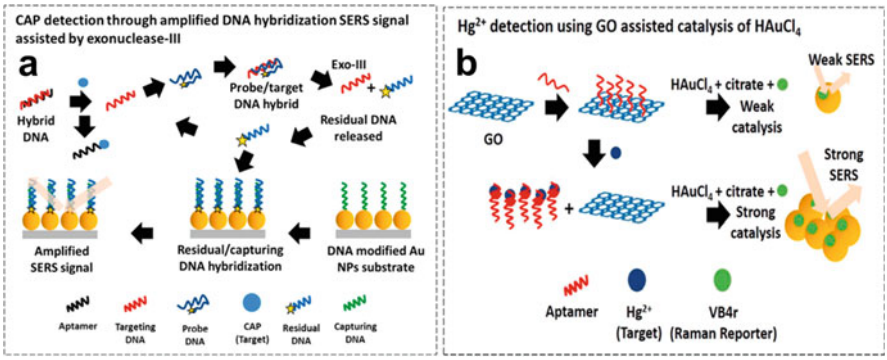

Fig. 6.5 (**a**) Chloramphenicol (CAP), the target molecule recognition process induces a structural change in biorecognition aptamer that is further enhanced by multiple rounds of DNA hybridization with Exo III. (**b**) An aptamer-functionalized sensor for mercuric ions detection uses GO to assist the $HAuCl_4$ reduction reaction. Adapted from reference Fang et al. (2019)

amplify the SERS signaling reporter molecules for indirect identification of mercury ions (Li et al. 2018b). The aptamer binds to the target Hg^{2+} specifically in their presence which leads to the inhibition of their further adsorption onto the GO surface. Due to this process, catalysis reaction of citrate and $HAuCl_4$ took place on GO surface that leads to production of AuNPOs that can be detected via SERS.

The sensitive detection of contaminants in food requires pretreatment to inhibit interference from nontarget molecules. Using gold nanoparticle conjugated with Raman tag 4-(mercaptomethyl) benzonitrile aptamers as Raman probes and AgNPs tagged cDNA-as signal enhancers, a new SERS-based aptameric sensor has been developed to minimize interference from nontarget species. When food samples contain acetamiprid, the target aptamer complex prevents the appearance of MMBN-AuNPs-aptamer-cDNA-AgNPs@Si, and the intensity of the Raman signal MMBN in Au-AgNPs@Si decreases. The real sample testing of the sensor was done to sense acetamiprid in apple juice with a detection limit of only 6.8 nm, which is expected to be used to detect traces of pesticides in complex food matrices.

6.6 Photonic Crystal-Based Sensors

Photonic crystals can be defined as the highly ordered nanostructures with varying dielectric constant and periodic scale of visible light wavelengths. Photonic crystals are nanomaterials and dielectrics having optical sensing features. Their nanostructures affect the movement of photons via periodic spatial modulation of refractive index. The repeated array of refractive index and Bragg reflections on

photonic crystals lattice structure lead to impeded propagation of light and culminate in the formation of photonic bandgap (Fathi et al. 2021). Analogous to flow of electrons in semiconductors, photons move in photonic crystals, and the hindrance in the propagation of photons in all directions led to full photonic bandgap. A pseudogap prohibits photon propagation in only some directions and results in incomplete photonic bandgap. The capability of photonic crystals to alter the spectral position at certain frequencies and the presence of photonic bandgap makes them really interesting and useful material for application in biosensors. Notably, there is no absorption of light in the process, and the photonic bandgap corresponds to the reflection of light via periodic arrays. In the simplest way, the optical sensing can be performed by monitoring the photonic crystal reflectivity shift or transmission spectra (Zhang et al. 2008). Photonic crystals such as liquid crystals, inverse opals, and fibers have been widely exploited for biosensing applications. Photonic crystal-incorporated biosensors have been used to detect a number of molecules like proteins, nucleic acids, pathogens, viruses, and cancer (Shafiee et al. 2014; Panda and Puspa Devi 2020).

Photonic crystal structures comprised of spatially organized periodic dielectric material that uniquely interacts with light, which results in high-efficiency reflection at specific wavelengths. Naturally, there are many photonic crystal-type nanostructures that exist (Vukusic and Sambles 2003). The most common examples are peacock, and *Morpho rhetenor* butterfly (Kinoshita et al. 2002; Zi et al. 2003). Apart from this, sea mouse, opals, and *Eupholus magnificus* also have geometrical patterns on the surface like photonic crystals through which light illuminates and reflects (McPhedran et al. 2003; Marlow et al. 2009; Pouya et al. 2011). On the basis of their geometry, the periodicity can be iterated in one, two, and three directions that implies the different dielectric constants. Thus, the fabrication of the photonic crystals can be done in one-dimensional (1D), two-dimensional (2D), or three-dimensional (3D) orientation (Fig. 6.6). Also, different types of materials have been used for their fabrication such as glass, polymer, silicon, colloids, and silk (Colvin 2001; Edrington et al. 2001; Jamois et al. 2003; Meseguer 2005; Freeman et al. 2008; González-Urbina et al. 2011; Kim et al. 2012; Han et al. 2012; MacLeod and Rosei 2013; Diao et al. 2013). Various top-down (electron beam lithography, thin film deposition, nanoimprint lithography, and electrochemical etching) and bottom-up (self-assembly) approaches have been utilized for the fabrication of photonic structures (López 2003; Kouba et al. 2006). Photonic crystals can be fabricated via various economic fabrication methods like colloidal self-assembly, mold-based replica printing, and hydrogels (Choi and Cunningham 2007; Yan et al. 2011; Fenzl et al. 2013). The advantages of photonic crystal-based biosensors over other techniques are cost-efficient fabrication and shorter assay time. Table 6.4 constitutes the faad safety sensors reported using photonic crystals.

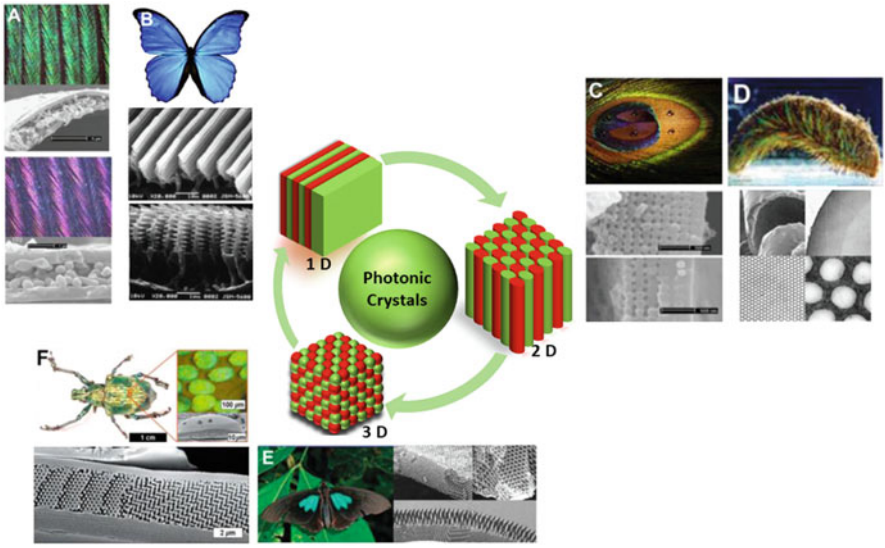

Fig. 6.6 Various examples of photonic crystals in nature constituting different colors: (**a**) one-dimensional neck feathers of domestic pigeons; (**b**) 1D, wings of ***Morpho*** butterflies; (**c**) two-dimensional, barbules of male peacocks; (**d**) 2D, iridescent setae from polychaete worms; (**e**) three-dimensional green spots in the wings of *Parides sesostris* butterfly; (**f**) 3D – *Lamprocyphus augustus* beetle (Chiappini et al. 2020)

6.7 Future Perspective and Conclusion

Optical biosensors are common analytical tools that can be utilized for point-of-care applications. Their small size enables their use in high-throughput applications to test wide array of samples in different conditions. Many optical biosensors are integrated with nanoparticles to enhance sensitivity or specificity. While developing an optical sensor for real-time detection, crucial factors to consider are simplicity, selectivity, sensitivity, robustness, and ease of use. All the discussed techniques have the potential to be practically employed in POC applications for food safety. Various optical sensors have been reported for food safety to detect various contaminants like toxins, pathogens, pesticides, and heavy metals. However, many sensors are in development stage and require substantial efforts before applying in real applications. The major challenge in the field of optical sensing for food safety is to develop the point-of-care sensors that are accessible and commercially available for resource-limited settings or remote areas so that illnesses or harmful effects due to adulterated and contaminated food products can be prevented.

Table 6.4 List of PC-based sensors for food safety

Target	PC used for detection	Technique	LDR and LOD	Real sample	Ref
Oxytetracycline	g-C_3N_4 nanosheet-modified tungsten trioxide film with inverse opal photonic crystals	Photoelectrochemical	1 nM to 230 nM; 0.12 nM	River water	Dang et al. (2019)
Mercury ion	1DPCs consisting of TiO2 and poly (N-isopropylacrylamide-acrylic acid), P(NIPAM-AA)	Fiber optic spectrometer	0 nM to 5 μM and 1 nM	Water	Xuan et al. (2016)
Lead and mercury	Colloidal photonic crystal hydrogel	Colorimetric	Lead: 1 nM to 1 mM; 1 nM Mercury: 10 nM to 0.1 mM and 10 nM	Tap water and lake water	Ye et al. (2012)
Mercury	Angle-independent photonic crystal	Colorimetric	1 nM to 10 μM; 1 nM	Tap and lake water	Ye et al. (2013)
Mercury and silver	Silica colloidal crystal beads and ssDNA-functionalized hydrogel	Fluorescence	10^{-9} to 10^{-2} M; 10^{-2} M	Tap water	Yan et al. (2017)
Ochratoxin A	Silica photonic crystal microspheres	Fluorescence	1 to 100 ng/mL; 0.02 ng/mL	Cereal	Li et al. (2021)
Ochratoxin A and fumonisin B1	Silica photonic crystal microsphere	Fluorescence	Ochratoxin A: 0.01 to 1 ng/mL; 0.25 pg/mL Fumonisin B1: 0.001 to 1 ng/mL; 0.16 pg/mL	Cereal	Yue et al. (2014)
Staphylococcal enterotoxin B	SiO_2 inverse opal photonic crystal	Reflectance spectroscopy	10^{-2} to 10^{3} pg/mL; 2.820 fg/mL	Milk and water	Shen et al. (2022)

(continued)

Table 6.4 (continued)

Target	PC used for detection	Technique	LDR and LOD	Real sample	Ref
Aflatoxin B1, ochratoxin A, and fumonisin B1	High-throughput photonic crystal microspheres	Fluorescence	Aflatoxin B1 and ochratoxin A, 0.1 pg/mL to 0.1 ng/mL;15.96 fg/mL and 3.96 fg/mL; fumonisin B1, 0.1 ng/mL to 10 ng/mL; 11.04 pg/mL	Wheat, rice, and corn	Yang et al. (2017)
Staphylococcus aureus and *Pseudomonas aeruginosa*	Gelatinase-responsive photonic crystal membrane	Reflectance	10 to 10^7 CFU/mL; 10 CFU/mL	Urine, lake water, and milk	Lu et al. (2022)

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