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NANOBIOSENSORS IN FOOD SAFETY AND QUALITY ASSESSMENT

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Contents

A. Abstract: 3
B. Introduction: 4
C. Methods 6
C1: Research Strategy 6
C2: Selection Criteria 7
D. Theory: 7
D1: Nanotechnology/Nanomaterials: 8
D2: Biosensors: 9
D3: Biosensors based on nanomaterials (Nanobiosensors): 11
E. Results: 13
E1: Applications of nanobiosensors: 13
E2: Nanobiosensors for food safety and quality assurance: 16
E2.1: Nanobiosensors for the detection of pesticides: 16
E2.2: Detection of pathogens/toxins in food products: 21
E2.3: Nanobiosensors for the detection of antibiotics: 39
E2.4: Nanobiosensors for the detection of heavy metals: 52
F. Limitations / Discussion: 57
G. Conclusions / Future Perspectives: 58
H. References: 60

A. Abstract:

The methods used to ensure food quality and safety are entering a new era. As a result, the food supply chain must be more efficiently intervened to safeguard the environment and public health and confront the challenges and current consumption trends. For example, analytical methods show various disadvantages, and microbial spoilage is currently ensured. Therefore, the science of nanotechnology is gaining more and more interest in the food and agriculture sector. New approaches enabled by nanotechnological tools, such as nanosensors for instant pathogen detection and antimicrobial food-contact packaging and surfaces, are proving their value for the food industry. However, applicability must also be ensured at the stage of development by evaluating the risks posed by using nanomaterials regarding both the environment and human health.

B. Introduction:

A significant section of the world's population is employed in the food and agriculture industry. Agriculture provides the raw material for industrialization and plays a crucial role in a self-sustained economy since it provides the essential ingredients to humans. However, insects, weeds, and pathogens significantly reduce yield. Humans control these factors using insecticides, herbicides, and fungicides. On the other hand, the increase in production leads people to increase heavy dosages of fertilizers and pesticides, thus contaminating water, soil, and food. Subsequently, when contaminated food is consumed, it leads humans to severe illnesses. However, health complications are not only caused by chemicals but also by the toxins produced by bacterial pathogens. Hence, food safety can only be ensured by detecting chemical and biological contaminants effectively. Many conventional and advanced analytical detection methods exist, such as spectroscopy, chromatography, molecular biology, culture plate, and immunology. However, they are either expensive and low sensitivity or time-consuming[1,2]. Humans and livestock use agriculture as their primary food source. However, various factors (biotic and abiotic) are limiting productivity and production. Therefore, food security also has to be maintained while food production is enormously increased for the needs of the fast-growing population[1,3,4]. Advanced technology is necessary for production to be increased while maintaining high levels of quality assurance, prevention diagnosis, and risk identification, thus achieving regional and global food security. Hence, to optimally utilize resources and improve consumer livelihood, affordable, real-

time, portable, and rapid technologies are needed in the food and agriculture industries[1,5,6,7].

The most promising technology that has been developed and has revolutionized the agriculture sector is nanotechnology[1,8]. Unfortunately, its practical application may be negligible at the moment. However, there are many future perspectives for improving conventional farming practices at every stage, from production all the way to post-harvest, thus enhancing the productivity of corps and, as a result, food production in general.

There are two different ways to increase productivity. The first is, by limiting yield losses caused by a large number of factors at all stages, such as diseases and insects (biotic stress) and also adverse environmental effects (abiotic stress), For example, harmful radiation, salt stress, nutritional deficiency, water stress cold stress, high-temperature stress, and avoiding post-harvest losses. The second is, employing advanced production techniques, thus minimizing input costs and leading to a high cost-benefit ratio. Both of these approaches can utilize nanotechnology to improve productivity and production[1,9].

One of the significant nanotechnology applications, nanobiosensors, is fabricated using miniaturization techniques, bioelectronics, electrode design, microfluidics, material science, nanolithography, and fabrication technology[1,10,11,12].

Biosensors are integrated self-contained tools used to characterize and sense biological materials. Their essential characteristics are currently being improved to be applied in various areas, including agriculture and food[1,13]. This review explores the applications of nanobiosensors in these industries, their potential and future perspectives, and the risks involved.

C. Methods

A thorough search was conducted in the published literature to extrapolate data for the use of Nanobiosensors in the food industry, and how nano-sized materials could be utilized towards food safety and quality assurance. The goal was to gather as much data as possible to help form a holistic, spherical view regarding established quality assurance methods, working mechanisms, safety concerns, current limitations and future perspectives.

C1: Research Strategy

A systematic literature search was conducted using the electronic databases PubMed, Google Scholar and Science Direct. The search strategy for each of the databases is depicted in Table 1.

Table 1 | Literature search strategy

Search Term	Search strategy	Citations retrieved (14.06.2021)	Studies retrieved after screening of title and abstract
Nanobiosensors AND Food Safety	Simple research, modified publication date from 2017 to present, PubMed	22	5
Nanobiosensors AND food quality assurance	Simple research, modified publication date from 2012 to present, PubMed	5	2
Biosensors AND nanotechnology	Simple research, modified publication date from 2017 to present	3.563	15

Nanobiosensors AND healthcare	Simple research, modified publication date from 2017 to present	34	5
Nanobiosensors AND toxicity	Simple research, modified publication date from 2017 to present	30	3

In addition, the reference sections of the retrieved studies were hand-searched for relevant studies.

C2: Selection Criteria

For the identification of relevant papers, the following criteria were applied.

Inclusion criteria:

Reviews, relevant books, newspaper articles

The paper had to include data regarding nanobiosensors

Publishing data not prior to 2012

Language: English

Exclusion criteria:

Confenerences' abstracts and letters

D. Theory:

D1: Nanotechnology/Nanomaterials:

Many fields of industry and science have incorporated nanotechnologies, such as the energy sector, healthcare, chemistry, bioinformatics, food engineering, medical science, environmental studies, bioscience, physics, biotechnology, food processing, electronics, and aerospace. Biosensor development has grown due to the ability to control and manipulate materials at molecular (nanometer range) and atomic levels, leading to an understanding of nanoscale-level fundamental processes. Nanomaterial characteristics, such as biological, optical, electrical, chemical, and physical, are determined mainly by dimensionality. Nanoscopic dimensions, for example, 0D, 1D, 2D, and 3D, are the basis of nanomaterial classification. When a material's three dimensions are nanosized, the nanomaterial is 0D NM (QDs and NPs). If two of the three dimensions are nanosized and one dimension is more extensive, it is 1D NM (nanobelts, NWs, NTs, nanoribbons, and NRs). If one of the material'smaterial's dimensions is nanosized, then it is 2D NM (nanolayers, CNTs, nanocoatings, nanodisks, nanoplates, nanowalls, nanosheets, and nanoprisms). Materials with no dimension in the nanoscale (bulk nanomaterials - > 100nm) are called 3D NMs (nanocoils, nanoflowers, nanopillars, nanoballs multi-nanolayers, and dendritic structures)[14,15,16]. The success of nanotechnology is primarily determined by synthesizing the nanomaterials, which enables their characteristic chemical, biological and physical properties. NMs have been synthesized by various strategies, such as the top-down approach (when nanosized materials are formed by restructuring a bulk material) and the bottom-up approach (the formation of nanomaterials atom by atom or molecule by molecule). The first approach uses various

techniques, such as chemical etching, laser ablation, lithography, and ion milling. The second usually includes the following techniques: chemical or physical vapor deposition and evaporation, molecular beam epitaxy, and chemical bioprocesses for supra-molecular complex protein-polymer nanocomposite and self-assembled monolayer production[16].

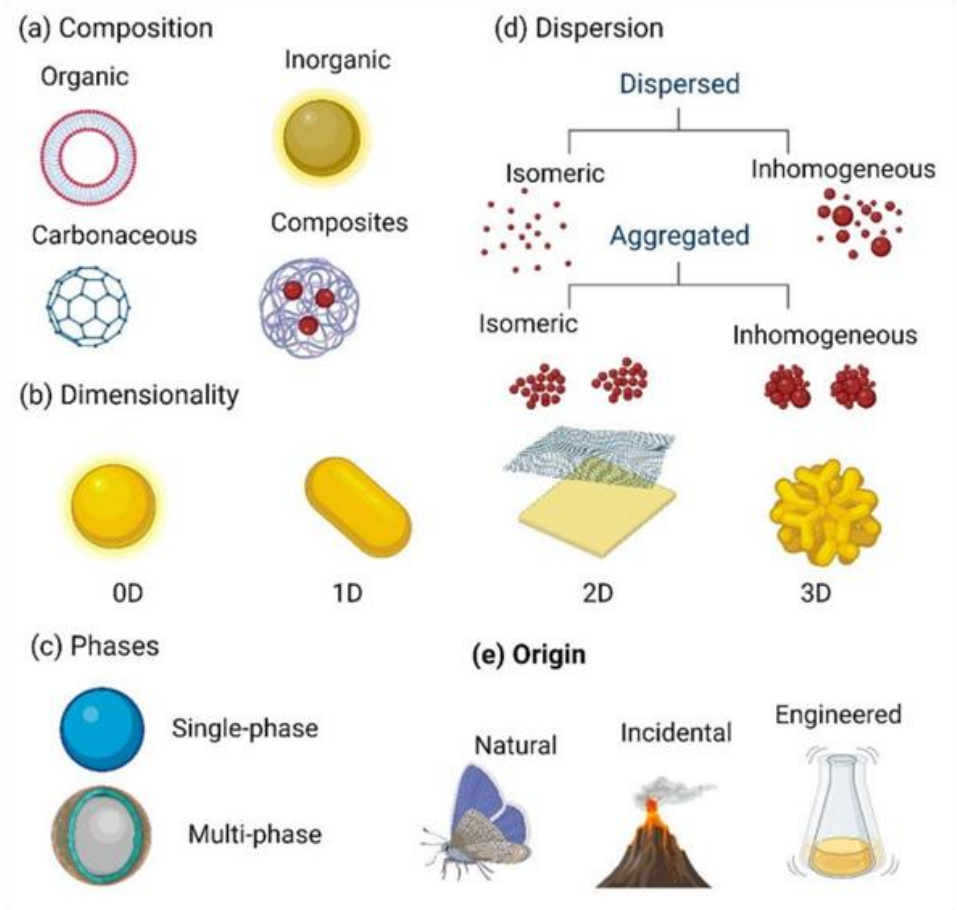

Fig 1: Various classifications of nanomaterials [17]

D2: Biosensors:

Biosensors are devices or probes with integrated biological elements, for example, antibodies or enzymes, and an electronic component for measurable signal generation. Various physiological changes, or the presence of biological or chemical materials in the environment, are detected, recorded, and transmitted by the electronic component. Biosensors have

various shapes and sizes and can detect low concentrations of toxic chemicals, pathogens, and pH levels. Typically, they comprise the parts described below[16,18].

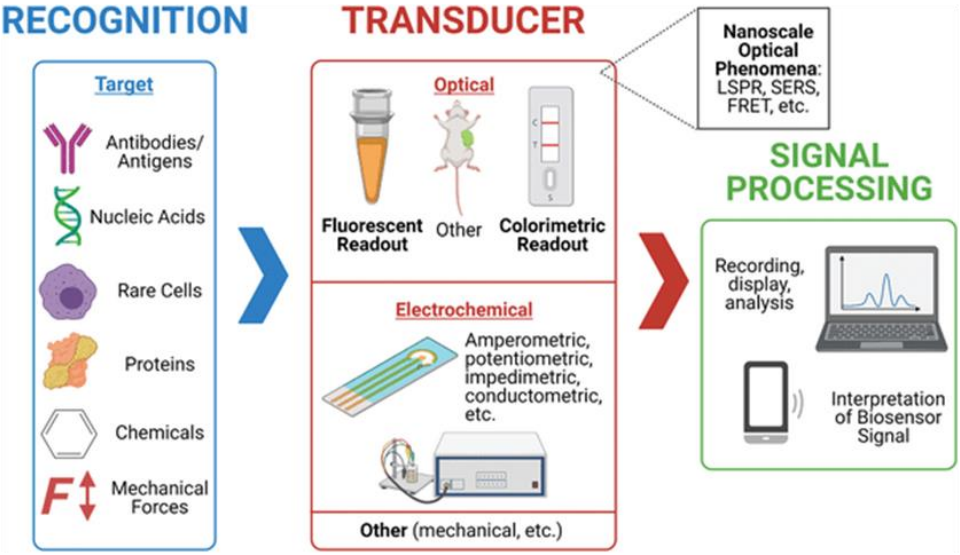

Fig 2: Basic biosensor components[19]

1. Analyte: The substance identified or detected.

2. Bioreceptor: A natural element or molecule that recognizes the analyte. When the bioreceptor interacts with an analyte, heat, light, antibodies, or pH signals are produced. This process is called biorecognition.

3. Transducer: A device that transforms one energy form into another. It is a crucial element since it translates the biorecognition event to electrical, measurable, and connected with the quantity or the presence of a biological/chemical target. This procedure (the conversion of energy) is called signalization. The transducers generate electrical or optical signals proportional to the number of interactions between the analyte and the bioreceptor. Based on the operating principle,

they are categorized as electronic, electrochemical, gravimetric, thermal, and optical transducers.

4. Electronics: The signal after its transduction is processed and prepared to be displayed. The transducer'stransducer's electrical signals are amplified and transformed into digital form. The display unit quantifies the processed signals.

5. Display: A unit composed of an interpretation system, for example, a printer or a computer, which creates the output for the corresponding response to be readable and understandable by users. The output can be a figure or a tabular, numerical, or graphical value, depending on the prerequisite of the end user.

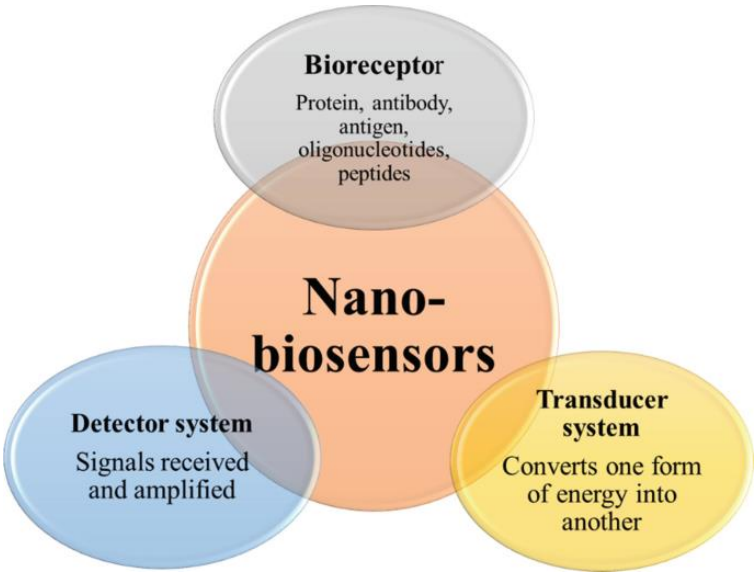

Fig 3: Schematic of nanobiosensor parts[20]

D3: Biosensors based on nanomaterials (Nanobiosensors):

Due to advances in nanotechnology, research and development regarding biosensors have become broader and multidisciplinary. The exploration of NMs, such as CNTs, NRs, NPs (oxide and metal-based, nanocomposites (dendrimers), QDs, and NWs for various characteristics, could enhance biosensor performance and increase the detection power through the control of morphology and size. Nanobiosensors work on the same principle lines as conventional micro- and macro-counterparts. However, nanoscale components are used for their construction to transform signals or data[16,21]. Dimensionality gives nanobiosensors an advantage compared to their conventional macro- and micro-counterparts since they are used for multidisciplinary applications. Nanobiosensors have great significance in nanotechnology since they can monitor chemical and physical phenomena in difficult-to-reach regions and perform medical diagnoses by detecting biochemicals in cellular organelles. They also conduct measurements of nanoscopic particles in the environment and industrial areas and can detect potentially harmful substances at ultra-low concentrations[16,21]. The involvement of NMs in improving biosensing has been widely researched based on the classification of NMs. For example, NP-based biosensors include sensors that use metallic NPs to enhance biochemical signals. Likewise, biosensors based on nanotubes that employ CNTs enhance the efficiency and specificity of reactions. Furthermore, the term "NW biosensors" includes biosensors with NW as charge carriers and transport. Similarly, QD-based sensors are also the sensors that utilize QDs as contrast agents to improve optical responses.

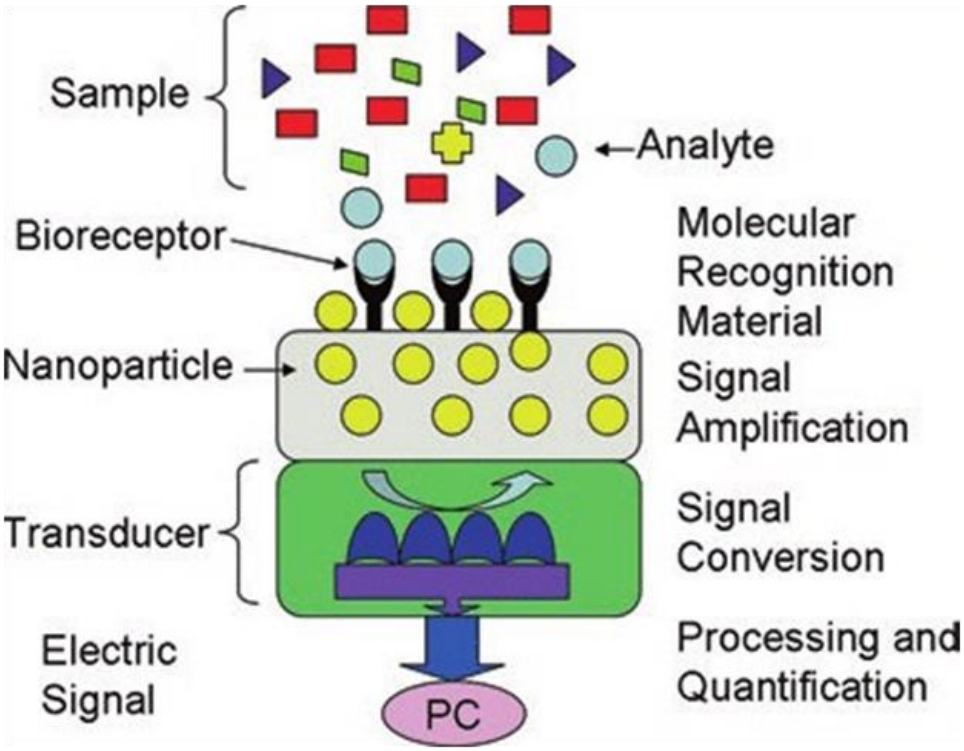

Fig 4: Biosensors using nanomaterials [22]

E. Results:

E1: Applications of nanobiosensors:

A fascinating potential field of application of electrochemical biosensors (potentiometric, impedimetric, amperometric, field-effect devices, conductometric, etc.) is the early-stage diagnostics of various cancer diseases[23,24,25]. Chronoamperometry (CA), square wave voltammetry (SWV), differential pulse voltammetry (DPV), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS) are highly sensitive, reliable, and also easy-to-use, and affordable techniques for the detection of cancer-related biomarkers[25,26,27]. Lab-on-chip biosensors are low-power portable devices that can be employed in cancer biomarker

research, possibly leading to clinical applications.[25,28,29,30,31,32] Electrochemical biosensors using surface nanoarchitecture offer several attractive features, such as low detection limits, robustness, and easy miniaturization. These biosensors also have small analyte volumes and can be used in turbid biofluids with fluorescing and optically absorbing substances.

Due to the enhanced performance of the electrodes, constructed with the nanomaterials, breast, lung, prostate, and other cancers can be early-detected by these biosensors. Moreover, nanomaterial-based electrodes are a reliable, inexpensive, and straightforward strategy for increasing reproducibility and sensitivity. Also, an up-and-coming future solution for cancer detection will be the employment of ultra-sensitive electrochemical nanobiosensors, which will offer very effective management and diagnosis of the disease[25].

Aptamer-based nanobiosensors are also being studied as potentially useful analytical tools in chemical analysis. They offer numerous advantages, such as simplicity, portability, fast response, and high sensitivity. Aptamers are biological recognition elements often used as biosensors due to their characteristics like high sensitivity, affinity and specificity, efficient immobilization, and small size. Nanomaterials have been used to develop aptasensors further, leading to the discovery of necessary, versatile diagnostic tools for various clinical applications[33].

Glucose sensing is critical for the prevalence of diabetes worldwide and food and drug industries. Novel strategies using nanomaterials with integrated fluorescent techniques have developed sensitive, superior glucose sensors. Fluorescent sensing is either minimally invasive or non-invasive. It is

susceptible, it can provide the micro-environment and structure of molecules, and the intensity and lifetime of fluorescence can be further utilized. Nanomaterials are used to develop fluorescent nanobiosensors for glucose sensing alongside biological components[34].

The coronavirus pandemic has underlined the need for effective methods of accurate detection and early diagnosis. Nanobiosensors are widely applied in biological detection, and among them, optical biosensors have gathered much interest due to direct readout and high sensitivity. Virus identification, rapid detection, serological studies, and genomic analysis have been achieved by optical nanotechnology. Optical nanobiosensors based on UCNPs, QDs, CL immunoassays, 2D-fluorescent organic molecules, nanoprobes, and noble metal NPs have been developed to detect pathogenic viruses. These nanobiosensors are categorized according to their detection mechanisms: chemiluminescence LRET effect, colorimetric analysis, NPs or molecules intrinsic luminescence, and plasmonic effect. All these strategies enhance sensitivity for both RNA and DNA detection. Some nanoprobes present detection limits down to the femtomolar level due to their elaborate design, a sensitivity level much improved in comparison to conventional methods[35].

Virus-induced carcinoma, caused by viral oncogenes, has to be detected early by point-of-care molecular diagnostics. On the other hand, high analytical performances cannot be combined with affordable diagnosis. A nanobiosensor has been developed to resolve this problem. This sensor is based on IDE and detects cervical cancer cells infected by HPV-16 via electrochemical impedance spectroscopy. The interdigitated electrode (IDE) chip surface is the basis for the interface between zinc oxide (ZnO) nanorods doped by gold (Au) coating

and HPV-16 viral DNA receptors. These biosensors have achieved excellent detection of HPV-16 E6 oncogene, the biomarker of cervical cancers caused by Hpv, due to the enhanced biocompatibility and sensitivity of the designed nanohybrid film. The nanobiosensor demonstrated a detection level down to 1M for the viral E6 gene target. Furthermore, a stable, functional life span of more than five weeks was also exhibited alongside good discriminatory properties against HPV-16 and satisfactory reproducibility[36].

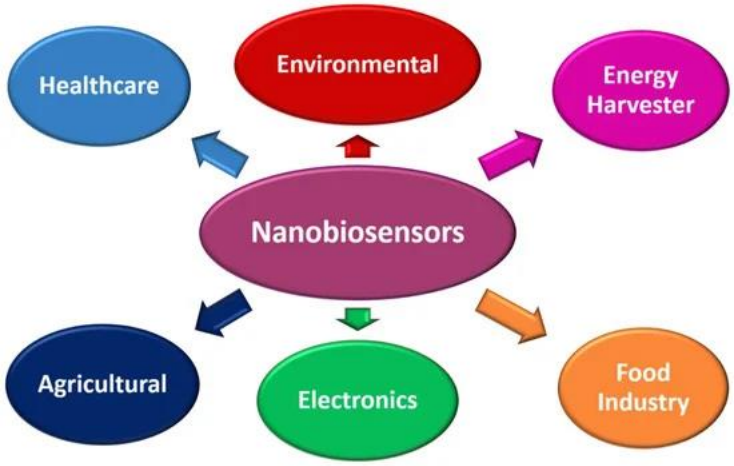

Fig 5: Schematic of several applications of nanobiosensors[37]

E2: Nanobiosensors for food safety and quality assurance:

E2.1: Nanobiosensors for the detection of pesticides:

Pesticides are commonly used in agriculture to minimize crop pest populations and increase productivity. Despite their usefulness, pesticides are potentially dangerous for both human health and the environment. Detection of their concentration can help determine the end product'sproduct's toxicity and minimize the overall use of pesticides since the overuse is a common practice.

Pesticides like parathion, methyl parathion, paraoxon, and fenitrothion can be detected by using various biosensor types. For example, multi – and single-walled nanotubes were used to fabricate enzymatic sensors with acetylcholinesterase and surface-plasmon resonance biosensors[38,39,40]. Also, the gold nanoparticle-based biosensor successfully detected chlorpyrifos and carbofuran at 0,06 and 0,08 μg/dm^3[39]. Carbaryl, methyl parathion, and monocrotophos have been successfully detected using nanobiosensors based on quantum dots, with immunofluorescent colloidal gold nanoparticles, sensing 2,4-D content up to 250 pg/L. Furthermore, nanobiosensors based on acetylcholinesterase with iron nanoparticles and chitosan have been developed[41]. Malathion was detected in tomato samples and pond water using voltage-based analysis, with a limit of detection of 0,3 mmol/L due to increased sensitivity[41].

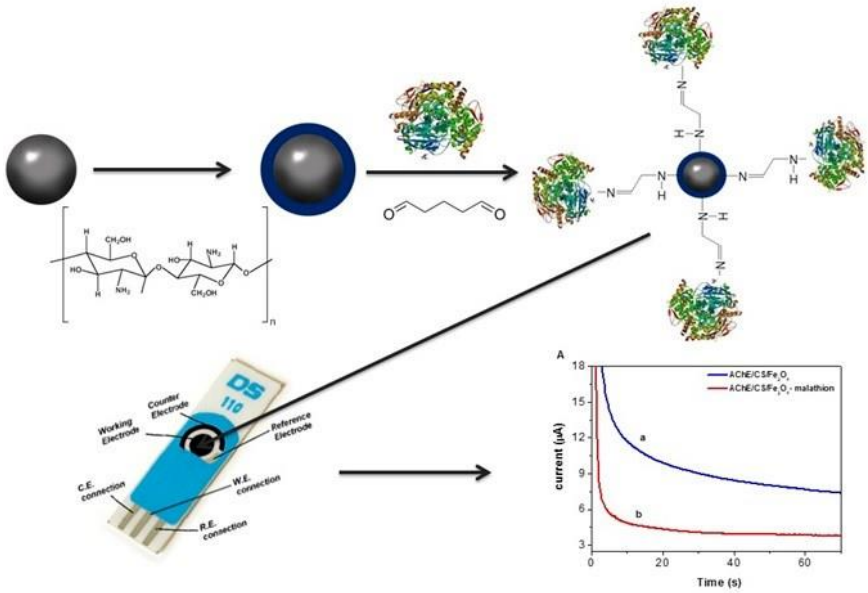

Fig 5: Iron nanoparticles and chitosan nanobiosensors[41]

Another example of an enzyme-based nanobiosensor consists of butyrylcholinesterase, tyrosinase, and alkaline phosphatase combined, using immobilized by origami paper, Russian blue nanoparticles. This nanobiosensor has been used to detect pesticides like paraoxon, 2,4-dichlorophenoxyacetic acid, and atrazine. It produced its signal using a potentiometric approach, thus strengthening the development of paper-based, portable nanobiosensors[42].

Electrochemical sensors can also detect various pesticides, insecticides, and herbicides. One example is a nanobiosensor consisting of hollow fiber pencil-based graphite with CuO nanoparticles and carbon nanotubes that detected in situ concentrations of glyphosate using voltammetry[38,43].

Carbon nanotubes are particularly popular for their applications in biosensors for food analysis and pesticide detection since they have interesting properties in electrochemical measurement and have a big active surface area at electrodes of smaller magnitude. Also, the electron transmission rate of carbon nanotubes and their sorption capacity is high. Therefore, they can also be used as electrode material for substrate immobilization on the surface. There are CNTs with one graphite sheet layer in a cylindrical tube (single CNTs) and CNTs where an array of single nanotubes with a concentric formulation create a nest (multi CNTs). Multi CNTs and microchips detect food additives like vitamins, sugars, flavors, and isoflavones. They could be used supplementary or even alone through an enzymatic biosensor method since it is cost-effective, fast, and straightforward. Enzymatic biosensors can detect carbamate pesticides by inhibiting acetylcholinesterase and its substrate, acetylcholine. The catalytic activity is inhibited by the carbamate, which binds to the enzymes' active site, blocking the Senne residue in the catalytic trial of

acetylcholinesterase through carbamylation or phosphorylation. A compact layer of polyaniline in the core-shell structure of CNTs is used to detect carbamate pesticides in vegetables and food. Through the immobilization of acetylcholinesterase on its surface, methomyl and carbamyl in samples can be quantified using chronoamperometry. [44]

Flexible sensors with robust sensing and low cost have been developed, based on graphene and terahertz, to detect pesticides at biological interfaces[45]. This sensor detects chlorpyrifosmethyl and chlorothalonil with detection limits of 0,13 mg/L and 0,60 mg/L, respectively. Furthermore, it has a lower cost due to fewer fabrication steps and is commercialized for pesticide detection[45]. A single-step analysis is the preferred method of analysis, compared to multi-step, due to the significantly reduced analysis time. Similarly, a nanocomposite nanosensor has been developed using mesoporous molecular sieves with carbon dots to create molecularly imprinted polymers for detecting kaempferol concentration in vegetables. Thus, anticancer properties can be further analyzed[46].

Furthermore, a tyrosinase-based optical nanobiosensor that detects dithiocarbamate has been patented, due to low cost and easy detection, since changes in optical properties can be detected by smartphone cameras. [38]

Pesticide residues are also present in the soil and can harm humans by affecting cholinesterase, thus leading to dyskinesia, a metabolic disorder. A new promising method for detecting organophosphorus pesticide residues with a limit of detection of 10-13 mol/L has been granted a patent[47,48]. It uses chitosan and silver-adapted nanoparticles on which acetylcholinesterase is immobilized. Another patented

nanobiosensor for pesticide detection is electrochemical. It consists of gold-palladium graphene quantum dot composite material and detects acetamiprid and chlorpyrifos with very high sensitivity and a detection limit of 0,37 fM. Acetamiprid acts on the synaptic site of the insects' nervous system and is used during storage for pest control[38,49]. Nanobiosensors can be used to detect acetamiprid, and they are considered promising alternatives. A highly sensitive nanobiosensor has been developed, with a limit of detection at 5,73 nM, which consists of aptameric DNA three-way junctions with three single-stranded DNA with G quadruplex sequences at the ends[50]. Graphene oxide is used for acetamiprid detection. This is the first nanobiosensor with a three-way junction that is used to detect pesticides. It also makes less noise due to the fluorescence quenching of graphene oxide; thus, it has excellent potential to be used in the food industry and agriculture[38].

Pesticides and toxic gases can be detected by SnO_2-based nanoparticles that have been used for sensing[16,51,52]. Another exciting category of biosensors used for pesticide detection is whole-cell-based biosensors. Compared to plant, or animal cells, they are rapidly proliferating and easy to use. They can interact with various analytes, display the transducer's electrochemical response, and transmit[53]. Due to high selectivity and sensitivity, they have been successfully applied in food analysis and environmental monitoring[54].

Water-soluble bi-conjugated QDs can detect pesticides, as well as bacterial toxins. Aqueous synthesized QDs have the advantages of longer photostability, stability, broad absorption, and highly-compatible and specific emission spectrum. If combined with a wide variety of biomolecules, an integrated, hybrid form of biosensors with unique magnetic and optical

properties with sensitive and specific detection abilities are formed. This is why QDs are particularly attractive as fluorescent probes for qualitative and quantitative analysis. Moreover, they can be arranged in an assembly to detect paraoxon, a toxic compound produced by the insecticide parathion, which they cover with layers of organophosphorus hydrolase, chitosan, and thioglycolic acid. Quantum dots are essential for bioanalysis and food safety since detection inhibits acetylcholinesterase, leading to acetylcholine accumulation in cholinergic synapses[16].

Pesticide detection is done mainly by measurement of Ach activity[53]. Au nanoparticle-based biosensors detect various pesticide molecules, like dimethoate, paraoxon, carbaryl, carbofuran, chlorpyrifos, etc., with a detection limit of 24µg/mL[54,55][54,55]. Lum-AgNPs integrated with H_2O_2-based CL detection generate specific CL "fingerprints"-characteristic for each pesticide. Also, a chitosan-TiO_2 graphene nanocomposite biosensor was successfully used to detect organophosphate pesticides in cabbage and was found to be highly stable and reproducible. The enzyme immobilization is exceptionally stable in this case due to the nanocomposite's porosity[16].

E2.2: Detection of pathogens/toxins in food products:

Biosensors require aptamers with molecule weights smaller than 25kDa for pathogen detection. Thus, they gain specificity since they bind to bacteria, viruses, proteins, molecules, and ions. A photoactivated indicator based on methylene dye has been developed for pathogen detection by measuring package oxygen. The photoactivated ink detects oxygen and serves as an indicator of aerobic pathogen growth. Pathogens like

Staphylococcus aureus can be detected by bacteriophage-based nanobiosensors approved by US Food and Drug Agency [1] .

Also, biosensors find applications in detecting various toxins in food products like processing contaminants such as acrolein, acrylamide, chloropropanol, polycyclic and biogenic amines, or preservatives such as methylimidazole, benzene, semicarbazide, and nitrosamines[38,56] .

Aflatoxins are a product of secondary metabolism in fungi. They can reduce the quality of several foods, such as rice, peanuts, and almonds. In addition, aflatoxins are categorized as carcinogenic since they cause hepatic carcinoma. Using the Plasmon resonance phenomenon, a portable nanobiosensor has been developed to detect these toxins[57]. This approach is used to measure the change in mass that is observed in the presence of aflatoxin. This nanosensor's detection limit is 2,5 ppb, and it has successfully achieved rapid detection of a spore-based miniaturized assay for aflatoxin in milk samples. Furthermore, it can be used for aflatoxin detection in almond, peanut, and rice samples. Some limitations regarding its use are the sensor's reusability and fabrication cost and the sample's quantitative analysis [1,21] .

Aspergillus flavus and aspergillus parasiticus can synthesize aflatoxins that cause growth retardation in children, carcinogenesis in the liver, and decreased immunological responses[38,58]. Permissible limits vary among countries. In the European Union, it is fixed at 2µg/kg, but in China, it is 20µg/kg, and in India, 30µg/kg. Recently, new trends in biosensing and the role that nanomaterials can have in aflatoxin detection were discussed[59]. Nanotechnological applications have offered increased reproducibility, sensitivity,

wide detection range, and limit of biosensors detection[60]. A comprehensive analysis was conducted regarding aflatoxins detection using a 0-dimensional electrochemical nanobiosensor. Nanosensors based on graphene, using gold nanoparticles, showed the limit of detection for aflatoxins in the range of 0,1-2,5 ng/mL [1] .

Various industries, including paper, leather, and textiles, use melamine (2,4,6-tri amino 1,3,5 triazine). It has also been used in milk to increase its false protein content because of high nitrogen elements. Fluorescence-based detection with DNA as a template was achieved using DNA-Cu-NPs, with ascorbic acid[61]. AS1411 templates are a novel approach for fluorescent copper nanomaterials. Fluorescence is quenched in the presence of melamine; thus, it can easily be detected, with a detection limit f 50 to 120 µmol/L. This is a highly sensitive, cost-effective method with less complexity[61]. These nanosensors are very useful in food safety.

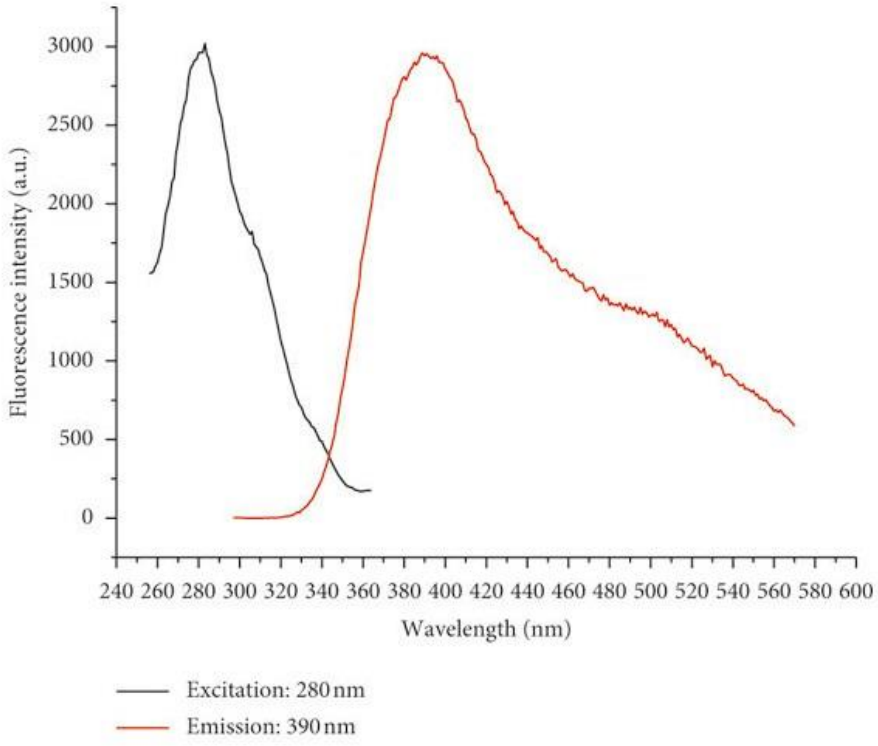

Fig 7: DNA-Cu NPs fluorescence spectra [61].

The mycotoxin ochratoxin A is potentially carcinogenic and is usually monitored by the expensive method of immunochromatographic assays. A chemosynthetic mimotope peptide that has been developed as a more affordable method can detect mycotoxin with the naked eye in 15 minutes, with a limit of detection of 0,187ng/mL[62]. Mycotoxins can lead to crop losses during storage; thus, they are a significant contaminant. Small nucleotides with fluaphore, called DNA aptamers, can be detected by FRET[63]. Zearalenone (ZEN) and ochratoxin (OTA) mycotoxins have been successfully detected by a graphene oxide-based, steganographic aptasensor, using Alexa fluor 488 aptamer and capture probe Cy3. Aptasensor's detection limit was 1,484 ng/mL and 1,79ng/mL for OTA and ZEN, respectively[61]. An optical nanobiosensor also detected

Zearalenone (ZEN) with a silicon dioxide layer using ZEN-specific antibodies. These antibodies were immobilized on a polyelectrolyte layer so that the refractive index changes upon mycotoxin binding, thus confirming its presence. The limit of detection was 0,01 ng/ml[64].

Toxins like ochratoxin and botulin, which cause serious medical problems like hepatotoxicity, neural paralysis, and cancer, are called collectively biotoxins. They contaminate food products during processing, storage, or packaging. Therefore, a chip for biotoxin detection has been fabricated and assigned a patent. This chip uses aptamers as molecular probes that bind with biotoxins and are modified using metal nanoparticles. This way, various toxins can be detected in food products with quick response and high sensitivity.

The performance of electrochemical sensors using nanomaterials and ways to improve it has been discussed[59]. Furthermore, electrochemical biosensors have been developed to detect toxins and foodborne pathogens produced by vibrio cholera and E. coli[38].

Significant outbreaks are also caused by Campylobacter jejuni, which contaminates food. Nanobiosensors for pathogen detection based on DNA aptamers are a primary method for food safety. 59-nucleotide single-stranded aptamers with high affinity have been developed to detect Campylobacter jejuni with a detection limit at 10CFU/mL. The results have been proven accurate by milk sample screening[65].

Until recently, pathogen detection in food demanded the use of cultural practices, which are time-consuming. However, a significant indicator of food spoilage is E.coli. Functionalized multi-walled CNTs and ZnO nanoparticles derived by sol-gel can

detect E.coli using β-galactosidase with a detection limit of 101 CFU/mL in 15 min[66].

Food poisoning is mainly caused by the foodborne pathogens E.coli and Salmonella. A concentrating method has been developed and patented, using diatomaceous earth as support, coupled with titanium and gold oxide, which can collect viable forms. This agent can be used for many microorganisms and was used to help capture 70%-80%microorganisms within 30 min[67]. In addition, another nanobiosensor has been developed and patented, which uses gold nanoparticles combined with single-stranded DNA molecules. This sensor is antibody specific for E.coli and Salmonella. Raman spectroscopy was used for the characterization of the samples. By implicating such nanobiosensor, foodborne pathogen detection can now be achieved more quickly and accurately[38].

A widespread threat to food industries is Salmonella typhimurium which causes Salmonellosis. Timely and rapid detection has now been accomplished with the help of nanobiosensors[49].

The rhizosphere and microbiome of plants is an indicator of crop health. These beneficial microbes increase nutrient absorption from the soil, subsequently increasing productivity. On the other hand, pathogenic organisms also inhibit plants. Thus, it is essential to extract and analyze polynucleotides. However, there are some significant problems that the conventional analytical methods present, like unwanted component coextraction with more dilution, due to which polynucleotide levels drop lower than the detection limit. Recently, a method for preparing extracts has been assigned a

patent and will likely result in better polynucleotide analysis[68].

Fungal pathogens like Sclerotinia and Fusarium cause rust diseases in plants. Nanobiosensors can detect impedance in DNA probes for such pathogens[60].

Microfluidics is the up-and-coming platform for analyte detection in the biosensor field because of its miniaturization, automation, and portability advantages[69,70]. With microfluidics, the flow of operations, which is precisely controlled, can occur in microfluidic channel networks, mixing, fluid transport detection, concentration, and separation. Thus, this help overcomes the matrix effect, a significant obstacle in the widespread use of biosensors for various applications, such as food complex. Industries and food handlers should easily detect the presence of pathogens in food products[71]. Microfluidic biosensors, nucleic acid amplification techniques, or other immune-recognition protocols are used to identify bacterial pathogens[72]. Nanobiosensors based on microfluidics is currently being developed to achieve point-of-care testing for instant identification of various foodborne pathogenic bacteria, with very high accuracy and sensitivity[70,72]. Engineered nanoparticles have been used in microfluidic detection for recognition, captivation, fictionalization, and concentration of bacterial pathogens in food samples since the biomolecule size is comparable[70,72]. Nano-techniques and other detection techniques, such as colorimetry, electrochemistry, fluorescence spectroscopy, LAMP, etc., are combined in microfluidic nanobiosensors, achieving off-chip or on-chip detection of several widely studied pathogens[73,74,75].

Electrochemical biosensors use electrodes to transform analyte concentration, or biochemical information in general, into an electrical signal which is analytically sound. The electrode is very important because it serves as solid support to immobilize biomolecules and electrode movement[75]. These biosensors are widely used in food pathogen detection and provide the advantages of low cost, high sensitivity, and potential for miniaturization[73]. Detection and amplification are combined in one miniaturized platform by integrating microfluidics with electrochemical measurement. Magnetic nanoparticles (MNPs) and immunoassays combined are often integrated with microfluidic electrochemical devices, aiming to provide compact analytical devices for pathogenic microorganisms in food products. Some of their privileged merits are common reagents, sample volumes, and rapid detection/separation[76]. Microfluidic biosensors using immunomagnetic separation (IMS) based on magnetic nanoparticles (MNPs) have enriched or isolated target pathogens from food samples[77]. Also, MNPs and catalases have been utilized to detect Salmonella typhimurium using a microfluidic biosensor with an on/off electrical signal. Various target bacteria were first separated and concentrated by MNPs altered with anti-Salmonella monoclonal antibodies, creating the magnetic bacteria. After that, the magnetic bacteria and catalases reacted with polystyrene microspheres (PSs) which were modified with anti-Salmonella polyclonal antibodies, thus forming the enzymatic bacteria. These are loaded in a PDMS microfluidic chip made of two outlets and two inlets with an integrated glass capillary. Then, a magnetic field captures them. Hydrogen peroxide was added after PSs were washed away, creating an oxygen gap in the glass capillary since it is catalyzed by catalases, thus creating the "off" electrical signal. This biosensor detects

Salmonella typhimurium in 3,7X101 and 3,7X106 CFU ml-1 and LOD of 33 CFU ml-1. The detection is completed in the time range of 2h.

Similarly, Yao et al. exploited MNPs-based IMS to develop a microfluidic impedance biosensor that detects the foodborne pathogen E. coli[78]. First, the MNPs modified with streptavidin isolated and concentrated the target bacteria. Then, gold nanoparticles (AuNPs) modified with aptamers against E.coli and urease were added. When they were incubated, they formed enzymatic bacteria. After the occurrence of enzymatic catalysis, biological binding amplifies the impedance signal. The signal was detected at a continuous flow condition by a combination of gold interdigitated array microelectrode with a microfluidic chip. The enzymatic bacteria supernatant was injected into the chip for the impedance measurement, and the detection was completed within two hours. E.coli was successfully detected within the dynamic range of 105 CFU/mL, with a LOD of 12 CFU ml-1. The biosensor can also detect other foodborne pathogens if specific bio-recognition elements are altered.

One of the most widely studied foodborne pathogens is Listeria monocytogenes. Using a similar method, Chen et al. conducted an impedance analysis based on MNPs-Listeria-GNPs-urease sandwich complexes and microfluidics for Listeria monocytogenes with urease catalysis[79]. Firstly, a separation chip captures the complexes, and then urea is added to form ammonium and carbonate ions. A microfluidic detection chip was then used, in which the resultant was transported, and the impedance measurement was performed to calculate the quantity of Listeria cells. This chip detected Listeria cells with a detection limit of 1,6X102 CFU mL-1 within 1 hour. An electrochemical microfluidic, disposable device was made by

Oliveira et al. to detect Salmonella typhimurium in a milk sample, using magneto-immunoassay, with AuNPs as a label[80]. 8-working electrodes (WE) consisted of the device, a counter electrode (CE), and a pseudo-reference electrode (RE). The LOD was 7,7 cells ml-1. The methods mentioned above integrate online separation and the detection of bacteria and could also be used to detect other biological targets.

Another good option for electrochemical sensing applications is graphene oxide (GO) since it has excellent electron transport capabilities, various functional groups on the basal plane and the edge, and a high specific surface area[81]. Nanocomposites based on GO, such as GO-carbon nanotubes (CNTs), offer controlled porosity and enhanced electroactive surface area to immobilize the biomolecules[81]. A microfluidic immunochip was developed by Signh, Ali, Reddy, et al. to detect Salmonella typhimurium using biofunctionalized GO-wrapped carboxylated, multi-walled carbon nanotubes (cMWCNTs)[81]. These composites act as transducer material and as coating on indium tin oxide (ITO) microelectrodes. GO-wrapped cMWCNTs offer enhanced electron transfer and a lot of functional groups, thus improving antibodies' loading and sensitivity to 0,376 CFUml-1. This method helped improve salmonella Typhimurium's sensing characteristics compared to other lab methods.

Furthermore, a new way was provided by this method for the fabrication of a portable electron device with high reproducibility and sensitivity. Their group also used a similar protocol later to design a microfluidic immunosensor with cetyl trimethyl ammonium bromide (CTAB) functionalized molybdenum disulfide (MoS_2) nanosheets (CTAB-MOS2-NS) for the detection of Salmonella typhimurium with a sensitivity of 1,79 kΩ/CFU-1mL cm-2 and LOD of 1,56 CFUml-1. Recently, a

thread-based microfluidic electrochemical aptasensor was reported by Jiang et al. It provides sensitive and rapid detection of the pathogen Vibrio Parahaemolytics in seafood[82].

The electrodes and the microchannels were fabricated using threads. The electrodes had the aptamer functionalized MoS2 nanosheets immobilized on their surface, achieving highly selective and sensitive sensing. This aptasensor selectively detected Vibrio parahaemolytics within the range of 10-10-6 CFU mL-1. This is a novel strategy for fabricating microfluidic chips and also electrochemical biosensors. Another effective strategy for improving the sensitivity of microfluidic biosensors, particularly for electrochemical microfluidic chips, is the enrichment of bacteria. This can be achieved by combining dielectrophoresis (DEP) for invasive and effective enrichment[83]. A multi-functional microfluidic chip was developed by Wang et al. by using interdigitated microelectrodes and also a micro-mixing zone to achieve impedimetric detection of several food pathogens[83]. Silver nanoparticles were also used to provide signal enhancement. The chip comprised a PDMS layer with a Tesla mixing zine and a detection zone channel. Also, a glass slide with gold interdigital microelectrodes. E. coli was successfully detected within one hour. Recoveries of 87,69%-110,86% and RSD of 6,3%-9,0% were obtained, and LOD under optimized conditions was 500 CFUmL-1.

An attractive point-of-care detection is colorimetric biosensors because they can easily detect target analytes by the naked eye, through color changes, or by simple optical detectors[73]. Therefore, they are widely studied, exploiting the advantages of the unique optical characteristics that nanoparticles have. For example, AuNPs provide a tunable color shifting when size and

shape change, corresponding to their aggregation status in solution and dispersion[84]. For this reason, they are capable of being used as colorimetric indicators. A microfluidic colorimetric nanobiosensor was fabricated by Zheng et al. to detect E. coli in chicken samples[85]. Polystyrene microspheres (PSs) were modified with capture and detection antibodies to achieve the immunoreaction, and AuNPs were used to indicate different concentrations of bacteria. HRP+H2O2+tyramine(TYR) was the mechanism on which the system was based. The microfluidic chip comprises two serpentine mixing channels to mix the nanoparticle/cross-linking agents and nanoparticles, a separation chamber to separate MNP-bacteria-PS complexes and the hydrogen peroxide, which serves as a catalyst, and also a detection chamber to investigate color changes. The nanobiosensor was used for the detection o E. coli-spiked chicken samples. An assay's duration was within one hour, and a LOS of 50 CFUmL-1 with a dynamic detection range of 5,0X100-5,0X104 CFUmL-1 was observed.

A microfluidic colorimetric biosensor was reported by Man et al. using gold nanoparticles (AuNPs) aggregated with thiolated polystyrene microspheres (SH-PSs)[73]. The chip's 3D structure consisted of a hose-based microvalve, a micro-mixing channel, a colorimetric detection chamber, and a reaction chamber. A simple, novel hose-based microvalve controls precisely fluid transportation. Thus enrichment, mixing, and detection can be achieved in one microfluidic chip. Conjugates of aptamer-polystyrene microsphere (PS) and cystamine were detection probes by allowing AuNPs' binding. AuNPs, aggregated on PSs, resulted in a visible change of color. The nanobiosensor showed a LOD of 6,0X101 CFUmL-1 in Salmonella typhimurium detection and recovered from 91,68% to 113,76% for spiked vegetables from salad samples.

For example, immune nanoparticles were used to develop fluorescent biosensors to target bacteria so that magnetic bacteria could be formed. Various fluorescent materials, for example, quantum dots or fluorescein, are used to conjugate magnetic bacteria to form fluorescent bacteria. Quantitative analysis of fluorescent bacteria's fluorescent signals leads to the determination of the amount of the target bacteria. Nanoparticles possess unique fluorescence properties compared to traditional organic fluorophores, thus permitting better signal transduction, simplified detection, and intensification. Microfluidic fluorescent biosensors have been exploited in foodborne pathogen detection, with detection limits as low as 101-103 CFU mL-1[70,75,86]. A microfluidic nanobiosensor for rapid detection of Salmonella in food products was reported by Kim et al[86]. The microfluidic chip, made of silicon, comprised a detection well, a serpentine channel (400µm X 50µm, wXh), an outlet port, and three inlet ports. Superparamagnetic particles mixed with sample solution are loaded for a concentration of pathogens and separation into the inlet port. Polyclonal antibodies against Salmonella and semiconductive fluorescent quantum dots were loaded on another inlet. Negative pressure was used to suck the two solutions so the meandering channel could be mixed and incubated. This resulted in a complex captured in the detection well by a magnet underneath it, leading to a fluorescent signal. A sensitivity of 103 CFU mL-1 was obtained for both homogenized chicken breast extracts and standard salmonella Typhimurium cells. Microfluidic nanobiosensors can also achieve multiple pathogen detection. A microdevice with integrated ZnO nanorods was developed by Yu et al. to detect multiple potential pathogens[75]. Six branched microchannels in a hexagonal arrangement make up the chip. On the ceiling,

herringbone-shaped structures provide enhanced mixing capability and multiplexed detection of up to six different pathogens. The chip performed the detection procedure based on sandwich immunoassay and immunological capture. Capture monoclonal antibodies conjugated with 3D nanostructured ZnO nanorod surface were immobilized on the chip's hexagonal sides. ZnO nanorods in a microfluidic platform were used in the study to help enhance the detection sensitivity since there are more binding sites for mAbs and less distance between the species and the surface. Target pathogens could be captured on the mAb-ZnO during the sample loading. Interfacing pathogens were washed out, and monoclonal antibodies labeled with biotin were added to form the sandwich immune complexes by reacting with the captured pathogens. A fluorescence signal of the final complex determined the concentration. The signal was generated by cyanine dye on the streptavidin from biotin interaction with streptavidin. A sensitivity of 3,6X103 EID50mL-1 was achieved in detecting H5N2 avian influenza virus. The above result was 20-fold higher compared to the conventional ELISA. Encoded microparticles fabricated with photocurable polymer and superparamagnetic nanoparticles (SNPs) were used by Kim et al. in a microfluidic device to detect foodborne pathogens[87]. The fabrication method for the microparticles was photopolymerization in a microchannel, and they had a chain-like arrangement following the external magnetic field's direction. Then, they were conjugated by a photo-immobilization process with capture antibodies using the linker. During the process, fluorescence-labeled biotin and streptavidin verified the linker function. The capture antibodies on the encoded microparticles reacted with pathogens in an assay for the first time. Detection antibodies were added after

washing and then reacted with the pathogen on the microparticles. The microparticles could be used to quantitatively analyze target pathogens after being decoded. With this method, four different types of pathogens could be simultaneously detected within 35 minutes: Staphylococcus aureus, Bacillus subtillis, Salmonella typhimurium, and Escherichia coli. The detection limit was 10-100 CFU mL-1. This simple method for multiplexed pathogen detection could also be extended to biomarker detection.

Portable, rapid, and low-cost assays for easy, in-field detection can be provided by fluorescent biosensors based on smartphones[70,88]. A fluorescence-based biosensor for high-sensitivity detection of Salmonella typhimurium with smartphone video processing was reported by Wang et al[88]. This method detects the target pathogen with a sensitivity of 58 CFU mL-1 in 2 hours. Microfluidic biosensors based on smartphones can potentially provide accurate, online detection of a single bacterium. On the other side, image capturing quality is a main limiting factor, as well as video processing speed and imaging post-processing procedures[89]. The performance of the fluorescent materials which label the target bacteria or bio-recognition elements can usually improve the sensitivity of fluorescence-based biosensors. Furthermore, faster and more accurate imaging and data analysis can be achieved using smartphones with high-quality data processing modules and camera resolution. In foodborne pathogen detection, microfluidic fluorescent biosensors provide fast speed, good specificity, and high sensitivity[88]. However, false positives and inevitable background noises still need to be addressed since the adsorption to substances in real samples cannot be avoided[90]. The binding of nanomaterials with a target leads to a change of resistance. Based on that principle,

chemiresistive nanosensors were recently developed, and they offer the advantages of easy, precise measurement, low cost, simple detection principle, and structure[91]. Electrochemical biosensors require redox species and a conductive medium, in contrast to chemiresistive ones, because no current flows through the medium, and the sensing event takes place in the substrate. The equipment required is simpler since only the measurement of conductivity or resistance is necessary to perform an analysis[92]. Carbon nanowire was used by Thiha et al. for a highly sensitive, rapid biosensing of foodborne pathogens on a microfluidic chip. Highly defined on-site suspended carbon nanowires were made in this study by integration of photolithography and electrospinning, using standard Carbon_Microelectromechanical Systems (C-MEMS) techniques. The label-free chemiresistive biosensing was conducted using an aptamer, and the assay was executed on a microfluidic chip. A salmonella-specific DNA aptamer probe was immobilized on the carbon biosensor before an assay. The carbon nanowire biosensor rapidly detected foodborne pathogenic bacteria in beef within 5 minutes, having a sample volume of 5µL only and LOD of 10 CFU mL-1. Good selectivity was also observed in detecting Shigella dysenteriae, klebsiella pneumonia, E. coli, and vibrio cholera suspensions. UV-vis spectroscopy is a well-established technique for quantifying RNA, DNA, proteins, and chemical substances. This method refers to the absorption/reflection analysis in the visible light and ultraviolet spectral region, and it is a technique that is well established[93]. E. coli bacteria in milk was determined by Lee et al. using UV-vis spectroscopy combined with magnetic nanoparticle clusters conjugated with antibodies in a 3D-printed microchannel in helical shape for capture and separation[94]. Then, a light absorption spectrometer was

used to determine the bacteria concentration. Antibody-conjugated magnetic nanoparticle clusters (MNC-EC) and free magnetic nanoparticle clusters (MNCs) complex were added in a sample solution during an assay and then put into the device, succeeded by a sheath flow. A flow of Dean vortices was secondarily formed when liquid moved through the curved microchannel. MNCs' dimensions were not as big as those of MNC-EC; thus, they could be separated because of the size-sorting effect that depends on Dean's drag force and the Dean's number. After separating the MNC-EC complexes, Escherichia coli concentration was detected using a UV-vis spectrometer. A LOD of 100 CFU mL-1 was obtained when the method was tested using a milk sample. A suitable for the identification of a wide variety of biological molecules and non-destructive measurement which provides fingerprint information is Raman spectroscopy[70,95]. There are numerous reports of Raman spectroscopic analysis used to identify foodborne pathogens[96]. A technique to enhance Raman scattering by molecules on nanostructures or metal surfaces is surface-enhanced Raman scattering (SERS), which leads to an increase in magnitude in Raman intensity[95]. A SERS-based microfluidic chip was developed by Mungroo et al. to discriminate food pathogenic bacteria by utilizing chemometric data analysis and silver nanoparticles[97]. Two solutions were loaded and mixed in a microfluidic chip with a silver nanoparticle-triton mixture and one with a bacterial sample matrix. Subsequently, they were fed into the SERS sensing window to measure the SERS spectra. The method could successfully discriminate eight species of pathogenic food bacteria (Salmonella enteritis, Salmonella typhimurium, Pseudomonas aeruginosa, Escherichia coli, Methicillin-resistant staphylococcus aureus (MRSA), Listeria innocua and Listeria monocytogenes) by using linear

discriminant analysis (LDA) and principal component analysis (PCA) on the spectral data. SERS will be widely used for foodborne pathogen identification since it is very efficient as a tool for food quality assurance, owing to its high sensitivity. However, the qualitative and quantitative analysis of food is still a great challenge due to the complexity of the samples. This emission is called luminescence, when a substance emits light after a biochemical or chemical reaction. Luminescence detection does not require a light source for excitation; thus, it is more straightforward than fluorescent assays[70,98]. A luminescence-detection-based immunomagnetic flow was developed by Lee et al[99]. The detection occurred in a 3D-printed cylindrical microchannel; thus, pathogenic bacteria could be separated and detected in a large sample volume. On the wall of the microchannel in the hollow cylinder, bound Salmonella bacteria and antibody-functionalized Fe3O4 magnetic nanoparticle clusters were magnetically immobilized. Sample solution and AbMNCs solution were sequentially loaded in the microfluidic separation during an assay. Successful binding of AbMNCs and Salmonella bacteria was observed since AbMNCs were concentrated magnetically in the microchannel. A rinse with PBS solution for one minute and luminescence measurements followed the reaction. The LOD obtained in the assay of Salmonella bacteria-spiked lettuce solution was 10 CFU mL-1, making it a sensitive and facile way to detect pathogenic bacteria which cause foodborne diseases. In addition, a detection mechanism that utilizes acoustic waves in acoustic biosensors can extract biophysical information from the analytes. A micro-nano-bio-acoustic sensor that detects Salmonella in milk samples, combined with LAMP, was developed by Papadakis et al[74]. This method has a LOD of 2 Salmonella cells/μL. CNT multi-layer biosensors combined with

microfluidic chip-based LAMP have been fabricated by Li et al. to detect E. coli by fluorescence analysis[100]. The LOD of the platform was 1 CFU mL-1. On the other hand, this method required more complex fabrication or fluorescence staining procedures and expensive equipment and consumables to achieve this kind of sensitivity.

Furthermore, false positive results due to the high sensitivity of LAMP are standard because of cross-contamination or carry-over during the experiment. In general, fluorescent and electrochemical methods usually do not directly identify microbes but rely on specific antibodies. One significant advantage of on-chip LAMP is its capability to detect various microbes using not antibodies but their specific gene sequences[70].

E2.3: Nanobiosensors for the detection of antibiotics:

Antibiotic residues in food products cause various health problems, and as a result, the permissible limits have been changed. More specifically, detecting sulfonamide residues in meat and poultry is critical[101]. Therefore, a nanosensor has been developed by He et al. for the detection of sulfadiazine in food residues, and it consists of an immunoassay labeled with nanoenzyme. Nanoenzyme conjugate has been fabricated with gold, platinum, silica nanoparticles, biomimetic bodies, and molecularly imprinted polymers. Different concentrations of the antibiotic sulfadiazine (0,5-12,5mg/L) were used to measure the sensor's accuracy, using enzyme-linked immunosorbent assay method for milk and honey, and high sensitivity was shown since the detection limit was IC15 0,09 and IC50 6,1mg[38,101].

Antibiotics given to pigs, poultry, and other animals are potentially harmful not only to human beings but also to the environment. One such antibiotic is Terramycin, and a novel method to detect it has been granted a patent. An aptamer of magnetic nanomaterial has been utilized to prepare the probes. The detection limit was 0.88ng/mL with less testing and fabrication cost. Also, other sensors have been patented to detect Kanamycin, using gold nanoparticles with aptamers combined with peroxidase enzyme. First, the aptamers are detected using magnetic isolation. Then, a colorimetric determination is produced at 450nm[38].

Similarly, a patent has been granted for developing a colorimetric method that detects oxytetracycline and Terramycin[38]. In addition, a nanobiosensor for norfloxacin detection was also fabricated. This sensor is based on fluorescence resonance energy transfer (FRET), utilizing core-shell upconversion nanoparticles (CSUNPs) to donate energy and graphene oxide (GO) to accept it. The detection limit was 0,47 ng mL^{-1}, which is lower than the commercial ELISA kit by 13-fold within two hours. This nanosensor can be used in food samples and veterinary medicine and can also be changed to detect other antibiotics if the aptamer is switched[102].

A portable and sensitive nanobiosensor for antibiotic detection in the raw milk of cows was developed. This system, which combines optomechanics, nanotechnology, and a spectral detection algorithm, can achieve sensitive detection of a variety of commonly used antibiotics, such as Oxytetracycline, Ampicillin, Sulfamethoxine, and Kanamycin. A detection limit of 0,25 times the MRL was reached with a linear range from 0 to 2 times the MRL for every different antibiotic[103].

A GO hydrogel-based fluorescence method was designed by Tan et al. The hydrogel consisted of physically mixed adenosine, GO sheets, and forming 3D macrostructures[104]. The fluorescent biosensor can, under optimal conditions, exhibit a linear range of 25-1000µg L^{-1} with a LOD of 25µg L^{-1}. Furthermore, this detection method was also applied for oxytetracycline in water samples and was proven highly accurate. Furthermore, nanomaterials like AuNPs and UCNPs can be integrated into fluorescent biosensors to detect antibiotics[104]. For example, a fluorescent biosensor based on aptamers was fabricated to detect chloramphenicol using UCNPs and MNPs[105]. The linear detection range under optimal conditions is 0,01-1,0 ng mL^{-1} with a LOD of 0,01 ng mL^{-1}. Moreover, the biosensor was applied to quantify chloramphenicol in samples of milk.

Fluorescent biosensors with high sensitivity were reported by Li et al[106]. These sensors were based on UCNPs and were used for Kanamycin detection. The UCNPs in this experiment acted as energy donors, and the graphene sheets were used as energy acceptors. The Kanamycin aptamer, modified with amine, could be attached to the UCNPs on EDC-NHS protocol. Kanamycin being absent, the conjugates are adsorbed on the graphene surface, thus bringing the energy donor and energy acceptor very close together, leading to UCNP emission. After Kanamycin binding with the aptamer, the aptamer's structure changes into the hairpin structure. Due to the low affinity of the hairpin structure to the graphene, the aptamer dissociates from the surface, blocking the FRET process. The LOD was as low as 9 pM and was observed to detect Kanamycin. Nucleic acid–related enzymes are incorporated into the detection process to improve sensitivity.

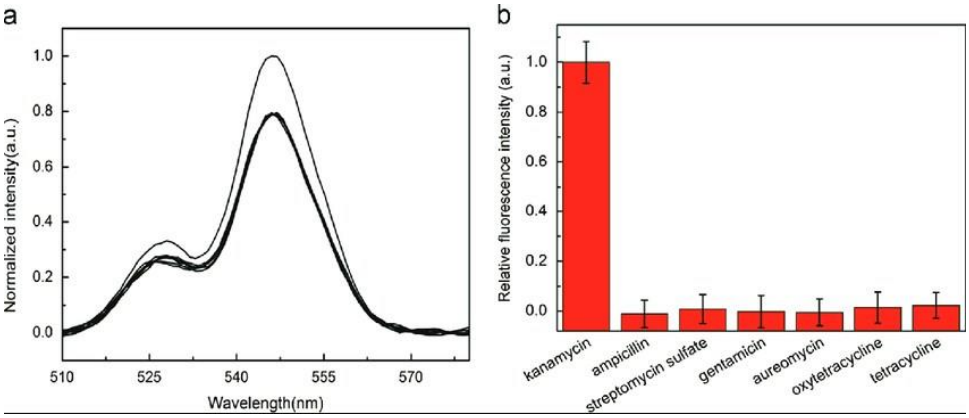

Fig 8: (a) Comparison between the spectrum in the presence of 0.1nM Kanamycin (highest curve), and the spectra of the sensor and those in the presence of other antibiotics (overlapped). (b) Relative fluorescence intensity of Kanamycin and other antibiotics[107].

A highly sensitive fluorescent aptasensor was proposed by Ramezani et al. to detect Kanamycin based on the activity of exonuclease III (Exo III), AuNPs, and dye-labeled complimentary strand stays on the surface of the AuNPs, leading to the emission of a weak fluorescence[107]. If Kanamycin is absent, a double-stranded DNA (dsDNA) is formed from the binding of the aptamer with its complementary strand. The dsDNA does not remain on the surface of the AuNPs. If Exo III is added, the aptamer is obtained again from the dsDNA, creating a cycle that results in solid fluorescence emission. The biosensor has a LOD of 321 pM.

Colloidal AuNPs are utilized for the development of solution–based colorimetric biosensors. AuNPs aggregation is used to detect the change of color. A colorimetric aptasensor was proposed by Emrani et al. based on AuNPs and ds DNA to detect Streptomycin[108]. Streptomycin being absent, the complimentary dsDNA strand which is dye-labeled, remains stable, thus leading to AuNP aggregation if NaCl is added. Subsequently, there is a visible change of color from red to blue. By adding Streptomycin, the binding of the aptamer with

its target leads to the removal of the dye-labeled complementary strand from the aptamer absorbed on the AuNP surface. In this case, the dispersion of the AuNPs remains stable when NaCl is added; thus, the aggregation with red color does not occur. The sensitivity of the aptasensor under optimized conditions is very high towards Streptomycin, with a LOD of 73,1 nM[108].

Abnous et al. reported a colorimetric sandwich aptasensor for the detection of chloramphenicol[109]. This aptasensor functions with an indirect competitive enzyme-free method. If chloramphenicol is absent, there is a binding of the AuNPs (colorimetric probes) to the well through a structure like a sandwich, for example, aptamer-biotin-streptavidin-biotin, resulting in vivid red color. With the addition of chloramphenicol, many biotin-modified aptamers washed out of the well. The few AuNPs that bind to the biosensor are 451 pM for detecting chloramphenicol. Another strategy using colorimetric biosensing is exploiting nanomaterial catalytic activity. Fe_3O_4 MNPs exhibit peroxidase-mimicking activity and are often used in this biosensor. Wang et al. reported a novel colorimetric biosensor to identify tetracycline based on Fe3O4 MNPs[110]. In this design, tetracycline molecules tend to form complexes with Fe(II) or Fe(III) on the Fe_3O_4 MNP surface, creating many N- and O- containing moieties. The Fe_3O_4 MNP complexes with tetracycline molecules can result in the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) catalyzed by H_2O_2, based on Fenton chemistry. Thus, a more visible color change is observed in the solution compared to the detection system where the tetracycline molecules are absent. This strategy is applied in tetracycline detection, with a LOD of 26nM, and doxycycline detection, with a LOD of 48nM. Furthermore, this method has achieved satisfactory results

when used to determine the content of drugs in oxytetracycline[111].

Some chemical reactions lead to light emission, which is called chemiluminescence. Generally, these reactions are fast oxidation reactions with multiple steps[111]. Detection based on chemiluminescence can be applied to various fields, mainly in optical biosensing systems. It is an effective and powerful analytical technique that offers many advantages: low-cost, wide dynamic range, operational simplicity, and high sensitivity[112]. Also, chemiluminescent biosensors integrated with nanomaterials have been widely studied. For instance, a novel chemiluminescent aptasensor was developed for detecting chloramphenicol in milk samples using gold flower-like nanostructures (AuNPs) to be the signal probes and MNPs to be the capture probes[113]. The MNPs conjugated with aptamers create bonds with the signal probes, as well as with chloramphenicol. The aptamer will bind to it after adding chloramphenicol due to more vital interaction. Thus, the unbound signal probes are freed towards the external magnet. Subsequently, the signal probe concentration decreases when the chloramphenicol concentration increases. This format has a linear range of 0,01-0,20ng mL-1 and a LOD as low as 0,01 ng mL^{-1} in a buffer. This method can be used for chloramphenicol detection in milk, and it is also easy to operate and highly sensitive.

Another method with high effectiveness is electrochemiluminescence (ECL). This method provides the benefits of electrochemistry combined with the high sensitivity of CL. The chemiluminescent reaction takes place at the electron surface. It is caused by electron transfer, leading to photons' emission in an excited state[114]. The applied potential controls the electrochemical process.

Various nanomaterials, such as metal nanoparticles, CNTs, silica nanoparticles (SiNPs), and quantum dots, have been utilized to enhance ECL biosensors' performance, making them a good option for developing biosensors for antibiotic detection[115]. An ECL biosensor with Ru(bpy)32+ -doped silica nanoparticles (Ru-SiNPs)/Nafion film-modified electrode for tetracycline detection was proposed by Chen et al[116]. An interesting finding was that the ECL response of the electrode was enhanced by the presence of tetracyclines. The linear range for tetracycline is 1-100µM, for oxytetracycline, it is 0,1-100µM, and for chlortetracycline, it is 1-100µM. The LODs are 0,23µM, 0,10µM and 0,16µM for tetracycline, oxytetracycline, and chlortetracycline, respectively.

Moreover, the biosensor showed high stability and repeatability, resulting in its application in the analysis of drugs. The oscillation at the interface of two materials is caused by photons and electrons and is called surface Plasmon resonance (SPR)[117]. Extensive attention has been drowned to SPR biosensors for the detection of antibiotics, owing to their real-time, label-free analysis, low cost, and compact design[118]. Fernandez et al. developed an SPR immunosensor using nanogold probes to detect antibiotic residues[119]. First, the active ester method was used to covalently attach the antibodies to the PEGylated nanoparticles to prepare the antibody-nanogold probes. Then, three biosensing strategies were developed to evaluate the biosensor's performance. The results showed a LOD of 0,07µg L^{-1} for detecting fluoroquinolone antibiotic residues. Also, a portable SPR biosensor with six channels, based on a plasmon of gold diffraction grating surface, was developed to detect various antibiotics in milk samples simultaneously. Sulfapyridine, enrofloxacin, and chloramphenicol, common antibiotics, were

used as models. The LODs under optimized conditions were 0,29 µg L^{-1}, 0,3µg L^{-1}, and 0,26 µg L^{-1}, respectively. Furthermore, the biosensor can detect antibiotics in milk samples, and clean-up steps are unnecessary. An SPR biosensor for the detection of amoxicillin was later developed by Yola et al. based on a molecularly imprinted nanofilm[118,120]. At first, allyl mercaptan was used to modify the chip's gold surface. The linear range under optimized conditions is 0,1-2,0ng mL-1, and the LOD is 0,022 ngmL^{-1}. Moreover, the nanosensor was applied to human plasma and chicken egg samples. Then, Sari et al. reported a novel SPR biosensor to rapidly detect erythromycin using molecularly imprinted nanoparticles[118,121]. By combining SPR techniques with mini emulsion polymerization and molecular imprinting, the biosensor was able to detect erythromycin within the range of 6,8-68,1µM, having a LOD of 0,4µM. The most frequently used and most common biosensors are the electrochemical ones. In this case, the electrical parameters of solutions can be affected by the alterations in the measurements of ions and electrons caused by chemical reactions. Electrochemical biosensors based on nanomaterials can be used to detect antibiotics since they have many advantages, such as easy operation, high selectivity and sensitivity, and low cost[118,122]. They are classified into voltammetric, amperometric, impedimetric, and photoelectrochemical biosensors based on the transducer type. The concentration of the analyte is linear to the electrons that are transferred. This is the principle behind the function of an amperometric biosensor[118,123]. Such biosensors evaluate oxidation or reduction flow amplitude during a fixed time at a given potential. Many nanomaterials have been integrated into amperometric nanosensors to improve the detection of

antibiotics. For instance, an amperometric immunosensor with carboxyl-functionalized CdS QDs was proposed by Kim et al. to detect chloramphenicol[118]. This sensor shows a linear range from 50 to 950 pg mL-1 and has a LOD of 45 pg mL^{-1}. It has also been used in milk samples to analyze their chloramphenicol content. A label-free amperometric immunosensor using graphene sheet-Nafion/thionine/Pt nanoparticles (GS-Nf/TH/Pt) modified electrode to detect Kanamycin by Wei et al[118,124]. The immobilization of the anti-kanamycin antibody on the surface of the GS-Nf/TH/Pt was achieved via electrostatic adsorption. The ability of GS and Pt to transfer electrons significantly improved the electroactivity of TH as a mediator for the transfer of electrons. Also, since Pt and GS have a relatively large specific surface area, the adsorption of kanamycin antibodies on the modified electrode is easy. Thus, the amounts of the loaded antibody are increased. Subsequently, the immunosensor's performance is improved, and it has a LOD of 5,74 pg mL^{-1} and an extensive linear range from 0,01 to 12,0ng mL^{-1}. Moreover, the immunosensor was utilized to determine Kanamycin in animal food products and showed satisfactory results.

Changes and interactions at a surface can be detected with high levels of sensitivity by electrochemical impedance spectroscopy (EIS). It is a label-free technique, able to achieve the extraction of information regarding the electrochemical features of a system in an effective way. Such information is the charge transport process, the double-layer capacitance, the solution resistance, and the diffusion impedance[118,125]. Due to its high levels of rapid detection times and sensitivity, EIS has played an essential role in biosensing various antibiotics[118,126]. An example of a biosensor is the label-free aptasensor with a screen-printed electrode (SPE), modified with a self-assembled conducting

polymer/Au NPs nanocomposite. The AuNPs that were incorporated into SPE increased the biosensor's performance considerably. The linear range was as wide as 0,05µM, and the LOD was 9,4 nM for kanamycin detection. An electrochemical immunosensor for tetracycline detection, using gold electrode-modified carboxyl Fe_3O_4 MNPs and a chitosan linker, was reported by Liu et al[118,127]. The MNPs were used to immobilize the anti-tetracycline monoclonal antibody on the electrode's surface and to accelerate the electron transfer. As a result, the resistance to the electron transfer is decreased for the MNPs modified gold electrode compared to the bare gold electrode. This decrease shows that the MNPs attached can ease the kinetics of the electron transfer and thus enhance the immunosensor's sensitivity. The fabricated immunosensor, under optimal conditions, shows a linear response to tetracycline concentration in the range from 0,08 to 1 ng mL^{-1} and a LOD of 0,0321 ng mL^{-1}. This immunosensor was used to detect tetracycline in the milk sample.

The principle behind voltammetry is the measurement of the current flowing that the working electrode produces. A solution with electroactive species contains the microelectrode; hence the analysis is performed by varying potential[118]. The analyte is identified by its voltammetric peak potential; thus, the method is highly selective and sensitive. Voltammetric techniques are divided into three categories: square-wave voltammetry (SWV), differential pulse voltammetry (DPV), and cyclic voltammetry (CV)[118,128]. All of these methods are commonly used in electrochemical biosensors for antibiotic detection. An electrochemical aptasensor utilizing the DPV technique was proposed by Qin et al[118,129]. The aptasensor was based on hierarchical nanoporous PtCu(HNP-PtCu) and functionalized graphene (GR-TH) and was used for kanamycin

detection. The composite of GR-TH served as a transfer bridge with a reasonable charge to ease the electron transfer rate. HNP-PtCu alloy immobilized more aptamers and promoted the kinetics of electron transfer. As a result, no redox peaks were observed at the bare GCE.

On the other hand, the modification of TH/GCE with HNP-PtCu increases the peak current. The aptasensor showed high sensitivity, and the linearity to Kanamycin ranged from 5X10-7 to 5X10-2µg mL^{-1}. The LOD was 0,42 pg mL^{-1}. Furthermore, the aptasensor was successfully applied to kanamycin detection in animal-derived food. An electrochemical aptasensor was also reported by Zhou et al. based on multi-walled carbon nanotubes (MWCNTS), exploiting both DPV and CV techniques to detect tetracycline[130]. The anti-tetracycline aptamer was immobilized by the carboxyl-functionalized MWCNTs, thus constructing the aptasensor. The electrochemical probe of Fe(CN)63-/4- was used to investigate the interaction between tetracycline and the aptamer.

A significant shape change was observed on the CV after modifying MWCNTs with the pretreated GCE. The shape of the CV was twice the bare GCE, which leads to the conclusion that the use of MWCNTs was critical in increasing the sensor's conductibility and the electroactive surface area. The linear range was $1X10^{-8}$ to $5X10^{-5}$, and the LOD was $5X10^{-9}$ M. This aptasensor was used in spiked milk samples to determine tetracycline. PEC oxidation combined with specific biorecognition creates the basis for the function of a photoelectrochemical (PEC) biosensor[118,131]. This biosensor offers the advantages of traditional electrochemical and optical biosensors, thus showing more outstanding performance than both. The privileged merits of PEC biosensors, such as easy miniaturization, ultrahigh accuracy, simple apparatus, reduced

background signal, and fast response, are the reasons for the growing attention towards them[118,132]. Functional nanomaterials have recently been incorporated to enhance PEC biosensors' performance in detecting antibiotics. A PEC aptasensor was proposed by Li et al. to detect Kanamycin specifically.

An aptamer was used as an element of biorecognition in the experiment, and water-dispersible graphite-like carbon nitride (w-g-C3N4)served as photoactive material. It was observed that the integration of GO into w-g-C3N4 (GO/w-g- C3N4 nanocomposite) improved the photocurrent response of the visible light. The linear range for kanamycin detection was 1-230nm, and the LOD was 0,2 nM. The PEC aptasensor also showed high stability and excellent reproducibility. A "signal-off" PEC aptasensor with BiOI graphene nanocomposite was reported by Yan et al. for oxytetracycline detection[118,133]. The BiOI produced a cathodic photocurrent signal in their study, and the anti-oxytetracycline aptamer was used as an element for biorecognition. BiOI produced a photocurrent response, able to be amplified by the doped graphene. When tetracycline was present, the specific binding of oxytetracycline to the aptamer decreased the photocurrent.

The linear range for oxytetracycline detection was 4,0 to 150nm with a LOD of 0,9nM. Another powerful technique with broad application in the biosensing field is surface-enhanced Raman scattering (SERS). It is a tool used to amplify Raman signals, by exploiting the advantages of nanoparticles, for example, silver nanostructures, gold nanostructures, etc[118,134]. In general, the SERS substrates adsorb on their surface the characteristic spectral signals stem from Raman nanotag, and then the localized SPRs are excited, resulting in signal enhancement[118,135]. SERS-based biosensors have

recently gained attention in the field of antibiotics detection due to their high rapidity, simplicity, and sensitivity. A SERS biosensor, for example, was proposed by Yang et al. for the detection of chloramphenicol accurately and sensitively[118,136]. The Raman reporter molecule was used to label the functionalized AuNPs. A competitive reaction occurs when chloramphenicol is present between the AuNPs and the free chloramphenicol molecules to conjugate with MNPs modified with antibodies. The biosensor was very selective and sensitive for chloramphenicol, having a LOD of 10pg mL-1 and a concentration range from 1 to 1X104 pg mL-1. The SERS magnetic biosensor could analyze chloramphenicol quantitatively in water, requiring no sophisticated sample processing. The concentration of specific analytes, for example, volatile compounds, small molecules, and cancer markers, can be measured by piezoelectric biosensors, which have found wide application for a long time. The piezoelectric effect in quartz crystal (QC) with no center of symmetry is the principle working behind it[118,137]. The lattice of the QC is deformed by applying pressure to it, forming a dipole moment in the QC molecules, thus creating a signal. Nanomaterial-based piezoelectric biosensors have been recently fabricated for the detection of antibiotics. For instance, a piezoelectric biosensor was proposed by Karaseva et al. utilizing nanoparticle molecularly imprinted polymers (NMIPs) to detect penicillins[118,138]. A receptor layer was formed at the piezoelectric chip's surface in a direct format by the NMIPs, which were obtained by precipitation polymerization. NMIP application could result in a mass increase upon binding, able to be analyzed if the resonance frequency of the device is detuned.

Furthermore, NMIPs can increase the area available for binding targets, leading to increased sensitivity. The linear range in this study was 0,1-0,5 µg mL-1 for penicillin G and 0,1-1,0 µg mL-1 for ampicillin. The LOD was 0,04 and 0,09 µg mL-1, respectively[118,138].

E2.4: Nanobiosensors for the detection of heavy metals:

Various nanobiosensors have been developed to meet the requirements of the food industry for detecting heavy metal residues[38]. Most bacterial and fungal diseases release certain toxins that affect human health and have chronic and acute impacts, like alteration in the metabolism of proteins, reduction of immunity, liver cancer, convulsions, and neurotoxicity. Optic, electrochemical, and piezoelectric sensors detect toxins in food products. Heavy metals are an example of such toxins. Clinical trials have shown that heavy metals like arsenic, mercury, cadmium, and lead can interfere with metabolic pathways, leading to profound health implications. For the detection of heavy metals in food products, new biosensors with green fluorescent signals and genetically modified bacterial cells were created. For example, a biosensor was created by Pola-Lopez et al. to detect the content of arsenic in the range of 5-140 µg/L[38,139].

A nanobiosensor was recently developed to detect Cd^{2+} ions in mussels and clams. This sensor is a colorimetric paper-based enzyme-coupled antimony tin oxide nanoparticle (ATONP) nanobiosensor with alkaline phosphatase (ALP) immobilized on ATONPs through 16-phosphonohexadecanoic acid (16-PHA). This sensor showed high selectivity for Cd^{2+} ions with a significantly low LOD of 0,006µg L-1 and a linear range from

0,005-1 µg L-1. Due to agricultural and industrial processes, cadmium is increasingly present in food and drinking water. This contamination leads to various health problems for both humans and animals. Therefore, an electrochemical nanobiosensor based on DNA was developed to detect Cd (II) ions in water samples. This method used a multi-walled carbon nanotube (MWCNT) and ethyl green (EG). The working electrode is formed by a glassy carbon electrode (GCE)/MWCNT, performing differential pulse voltammetry (DPV) analysis to detect Cd(II) ions. The working electrode has ds DNA immobilized on its surface, and its presence reduced the reduction peak current of the indicator dye EG, which binds preferably to ssDNA. The interaction of the Cd(II) ions with the dsDNA leads to an unwinding of the dsDNA to ssDNA, followed by binding of the EG molecules, thus producing a higher reduction peak current, and more specifically, its proportional to its concentration. This method achieved a detection limit of 2nM (less than the limit set by WHO as permissible for human exposure, a linear detection range from 2nM to 10nM, and a sensitivity of 5nA nM^{-1}[140].

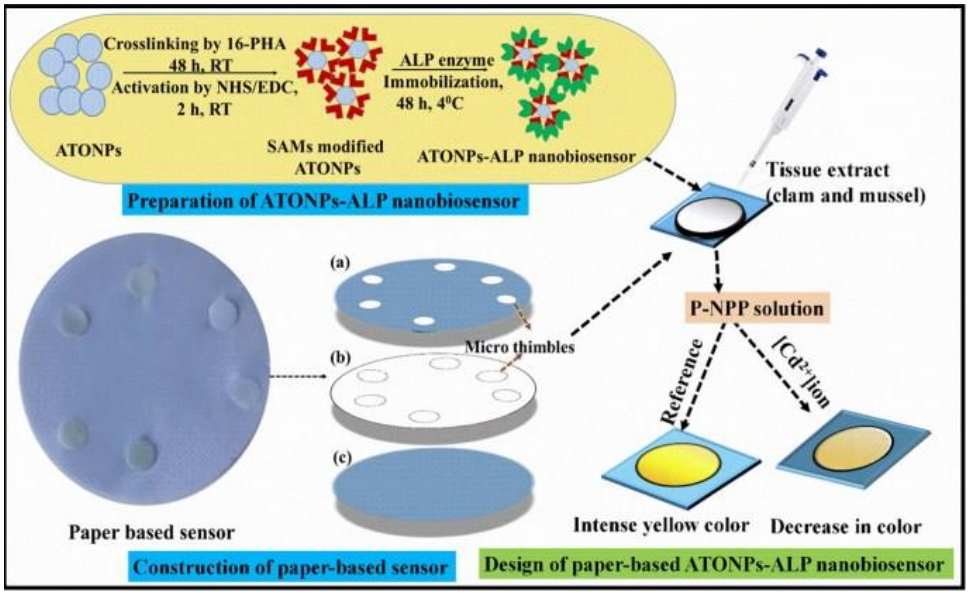

Fig 9: Miniaturized ATONP-ALP nanobiosnsor, integrated in the paper-based format, for Cd^{2+} colorimetric detection[140].

Various adverse effects on human health are also caused by mercury (Hg)[141]. Recently, the levels of Hg2+ have been successfully determined by various methods such as AAS/AES, ICP-MS, and nanobiosensors. This methods, though, require complex sample preparation and expensive equipment. Nanozymes seem to be able to resolve some of these issues by developing platforms for sensitively detecting Hg2+ and other heavy metals[141]. Cui et al. fabricated a fluorescent biosensor based on the reaction between bisthymine (T-T) and Hg2+[141,142]. T-T mismatch binds specifically with Hg2+ since it is a Hg-specific oligonucleotide. This carbon dot (CD)-tagged oligo-deoxy ribonucleotide (ODN) and GO-based nanobiosensor functions as a Hg2+ quencher with a LOD of 5-200nmol L-1. Another nanobiosensor for Hg2+ detection was developed by Wordofa et al. using DNA-modified single-walled carbon nanotubes (SWCNTs)[141,143]. The label-free chemiresistive nanobiosensor utilized the release of poly-A upon T- Hg2+-T formation to detect an alteration in resistance, which was used to calculate Hg2+ levels. This nanobiosensor exhibited a LOD within 0,5-100nmol L-1 for CH3Hg+ ions. AuNPs and ssDNA

oligonucleotides can also be used for Hg2+ detection. They were utilized in the fabrication of a colorimetric nanobiosensor by Memon et al[141,144]. The fact that T- Hg2+-T domain cannot attach to the AuNPs as a dsDNA results in the NPs agglomeration, which alters the hydrodynamic particle radius and can be detected using dynamic light scattering. Hence, the authors suggested a rational assembly with significantly improved sensing sensitivity. A linear range from 50 to 200nmol L-1 was observed for Hg2+, and the LOD was reduced at 15nmol L-1. Oligonucleotides are capable of achieving sensitive Hg2+ detection. However, more research is needed to resolve the problem of complexity regarding the fabrication of oligonucleotide-based nanobiosensors and the need for more diagnosis due to probe and target mismatch.

Hg2+ and Au exhibited a binding affinity towards thiol groups. A voltammetric nanobiosensor was fabricated by Asadpour-Zeynali and Amini to determine Hg2+ levels, utilizing a hydroxide NP-improved electrode intercalated with mercapto-carboxylic acid, with a LOD of 0,8 nmol L-1[141,145]. A GCE/rGO-SH/AuNPs electrode was designed by Devi et al. utilizing an rGO-SH loaded with GCE, with a LOD at 0,2µM for Hg2+ detection[141,144]. Another selective and feasible strategy was developed by Sharma et al. to optically sense Hg2+ in water using AgNPs with different pH, functionalized with thiol-terminated chitosan (Ch)[141,146]. The interaction of the Ch-AgNPs with Hg2+ led to a change of color of the nanoparticles, and the LOD was 5µg L-1.

Other moieties, such as proteins and peptides, aptamers, polymers, and cysteine, can modify the surface of nanomaterials, leading to selective binding of Hg2+, thus enhancing the transduction of signal based on the Hg2+ level. However, all require advanced analytical devices and complex

sample preparation of these approaches. The truth is that those expensive and time-consuming procedures are necessary for increasing the sensitivity and selectivity of the NPs by using different moieties for fictionalization. Furthermore, ion interference might occur and has to be overcome via coupling formation among functional groups. Therefore, platforms based on nanozymes were proposed to address these disadvantages since they are highly selective and sensitive, affordable, and simple sensors based on nanomaterials for on-site detection of Hg2+[141].

Conventional methods are still more precise, accurate, and sensitive in the determination of the levels of Hg2+ compared to nanomaterials. However, the selective framework-based sensor for detecting Hg2+ can be improved by a covalent organic framework (COFs) with various recognition methods such as solid-phase extraction, chromatography, and membrane separation[141,147]. Strong, covalent bonds form three- or two-dimensional porous crystals used in this technique. The structure of COFs is characterized by symmetry and geometry. Proper topology, large reaction areas, well-organized channels, symmetry, configurable sponginess, expectable structure state, optional building blocks, easy process, and chemical and thermal stability are merits that can be potentially helpful for sensing systems[141,148].

Peng et al. employed core-shell Au@Pt NPs for the colorimetric detection f both Hg2+ and Ag+ simultaneously, with LODs of 2.0nmol for Ag+ and 3,5 nmol L-1 for Hg2+[141,149]. Also, polyvinyl pyrrolidone (PVP)-functionalized PtNPs were used by Zhao et al. for simultaneous detection of Ag+ and Hg2+ levels with LODs of 9,75 nmol L-1 and 17,75 nmol L-1, respectively[141,150]. A bismuth oxy-iodide nanosystem was fabricated by Hsu et al. as a nano-network for efficient and

selective Pb2+ and Hg2+ sensing at nmol L-1 levels[141,151]. A molybdenum (IV) selenide nanozyme functionalized with biosynthesized chitosan was developed by Huang et al. for Hg2+ colorimetric determination. Hg2+ activating effect on the enzyme performance through the reduction of Hg2+, which is adsorbed by chitosan, is the basis of this sensing system[141,152]. Hg2+ levels can be determined selectively with a LOD lower than 5 nmol L-1 by employing TMB as a colorimetric index. Also, CS-MoSe2NS can be integrated with a smartphone, with a LOD lower than 10 nmol L-1 for Hg2+ detection[141,152]. This system showed improved applicability in actual samples and high levels of selectivity, thus being a promising platform in the field of portable, biocompatible nanozymes for Hg2+ determination in various samples such as serum, food, and water[141].

F. Limitations / Discussion:

The possible application of nanobiosensors in the food industry might be promising. However, there are still many challenges. Toxicity and ecotoxicity of nanomaterials owed to their uniqueness (composition, structure, surface-to-volume ratio, size, etc.) are major concerns linked to their application. Hence, their environmental impact should be precisely assessed regarding retention time, dose, immune response, size, and accumulation process[38,153]. Another limitation that needs to be addressed is miniaturization for the nanobiosensors to be portable.

If, for example, nanobiosensors are integrated with information technology, the food/agricultural industries in remote areas can improve their productivity, lower costs, understand disease outbreaks before onset, and utilize natural resources (climatic

conditions, soil, and water). Also, the fabrication cost of nanobiosensors needs to be reduced. This aspect can be improved using alternative biological components at a lower cost. Examples are cells/enzymes and novel matrices for nanomaterial immobilization and stabilization, for instance, chitosan, to enhance the nanobiosensor's reusability and stability[38,154].

Another challenging area regarding nanobiosensors is transforming a prototype into a product that can be commercialized. The reason is that field-scale trials need to estimate and calculate nanobiosensors' overall performance in actual implementations. Moreover, the end users must also be familiarized with nanobiosensors. Also, the strict regulations implemented in the food industries must be followed. Recently, it was suggested that the use of nanobiosensors by some manufacturers remains covert since the consumers' acceptance is not sure, and there is fear that it might lead to consumer-manufacturer disagreement[38,155].

G. Conclusions / Future Perspectives:

The use of nanobiosensors by food and agriculture industries is rapidly becoming wider, improving productivity and utilization of natural resources, thus making this sector more sustainable. Nanobiosensors can be used not only for the efficient detection of pathogens and harmful chemicals and adulterants but also for the assessment and pH of the soil, evaluation of moisture, and disease management. The agriculture and food industries can be the place to start their application until they finally reach the stage of commercialization. A small number of

industries, for instance, Nippon, IBM, and Roche fabricate nanobiosensors for various applications. The number of available reports regarding commercialized nanobiosensors in the food and agriculture industries is still insignificant. However, there are some available reports regarding commercialized nanobiosensors in medical applications and diagnostics. However, the cost for the fabrication of nanobiosensors, evaluation results, automation trials, and validation of field trials necessary for prototype miniaturization to enter the industry for production is still very high. In addition, the market currently cannot bear and offset these expenses, which is the reason for the lack of commercial nanobiosensors in the market. A more cost-effective strategy is the extraction of novel nanomaterials from waste biomass. Another essential aspect that needs further investigation is nanobiosensor versatility. The development of a nanomaterial array, for the detection of various materials, according to bioassays, may lead to the increased commercialization of portable nanobiosensors.

Smart agriculture and precision farming are potential future perspectives that the integration of GPS systems and nanobiosensors can achieve. Thus, the farmers' decisions regarding fertilization, harvesting, irrigation, and pest control will be better, and minimal natural resources will be used. In conclusion, highly sensitive and specific, customized nanobiosensors are a realistic expectation for the near future.

Fig 10: Smart nanobiosensors in agriculture [156]

H. References:

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