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*Review*
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# Recent Trends in Design and Development of Nanomaterial-based Aptasensors

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**Abstract:** The past decade has witnessed extensive research in the field of biosensors, where nanoaptasensors achieved widespread interest. Aptamer, the single-stranded DNA or RNA nucleotide, is extensively employed as a bioreceptor due to its stability, ease in a modification that ensures convenient immobilization strategies, reducing the cost of sensor manufacturing. The detection limit and sensitivity were notably improved when modified with nanomaterials such as metal nanoparticles, carbon nanomaterials, graphene, quantum dots, and other nanocomposites. This paper introduces various design strategies for the fabrication of sensors utilizing aptamers and nanomaterials in - developing signal-readout mechanism, focusing mainly on the latest research in food, biomedical and environmental applications. Aptasensors that utilize various signal recognition methods such as electrochemistry, colorimetry, luminescence, and fluorescence are highlighted. This review offers a wide range of outlook for future developments of aptamer bioreceptors employing the unique physicochemical nanomaterials as efficient transducers and amplifiers. Furthermore, this review will also provide an insight into aptamer-aided biosensors' technology over the last three to five years of work.

**Keywords:** aptasensor; nanomaterial; nanoaptasensor; aptamer; electrochemical; optical.



## 1. Introduction

Aptamers are single-stranded synthetic oligonucleotides that can fold into 3-dimensional shapes upon binding with a target molecule. Aptamers can act as molecular probes where they can bind non-covalently with high affinity and specificity to a target molecule of any choice, such as biomolecules (protein, lipid, DNA, and RNA), and biological cells (blood cell, virus, and bacteria) [1]. Aptamers are generated via an in vitro process known as Systematic Evolution of Ligands by Exponential enrichment (SELEX) [2]. As aptamers with high binding quality and specificity cannot be easily produced by the conventional SELEX process, various improvised methods involving further refinement and optimization are being used worldwide to ensure the quality. Sequence optimization via *in silico* sequence recombination and *in vitro* functional evaluation have been used to enhance the binding

affinities. However, both methods are flawed due to the hydrophobicity of the base part of nucleic acids. Hence there is a need to improve the binding using various techniques such as high throughput sequence optimization via DNA microarrays, stabilization of aptamer structure by locking nucleic acid, and introducing hydrophobic moieties into aptamers, and multivalent interaction of aptamers [3].

Aptamers are attracting a great deal of attention in developing the novel specifically recognized biosensor element known as the aptasensor. Aptamers are replacing antibodies readily as target receptors for their superiority. Considering both are chemically equivalent for their affinity and specificity, the windfall for aptamers comes from being smaller than antibodies, favoring giving both higher stability and reversible structure-switching abilities [4,5]. These benefits undoubtedly overcome the advantages of antibodies providing new opportunities making aptamers much more appealing. Figure 1 shows the increasing number of publications generated in the field of aptasensors in the last 10 years.

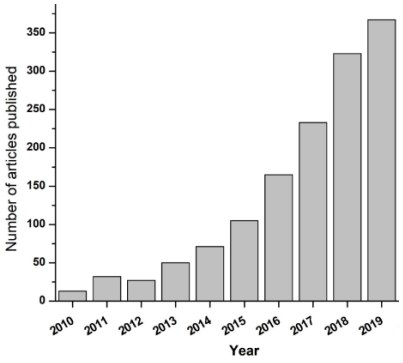


**Figure 1.** Aptasensor publication trend in the last decade when searched with keywords aptasensor and nanomaterial (data obtained from science direct).

In producing biosensors, numerous exercises have been done in utilizing aptamers as both capture and detection probes. As a result, researchers have identified a plethora of targets ranging from small molecules to macromolecular target analytes such as proteins, peptides, amino acids, antibiotics, toxins, and cells while producing their respective aptamers. On top of that, modifications can be easily achieved as aptamers are flexible and conveniently conjugated to various derivatives. Figure. 2 illustrates the major components used in designing aptasensors as a detector, including targets, bioreceptor, and transduction elements. Although countless efforts have increased the publications on aptamers, detailed research reports are still not enough. This review only focuses on the work with similar targets while using antibodies.

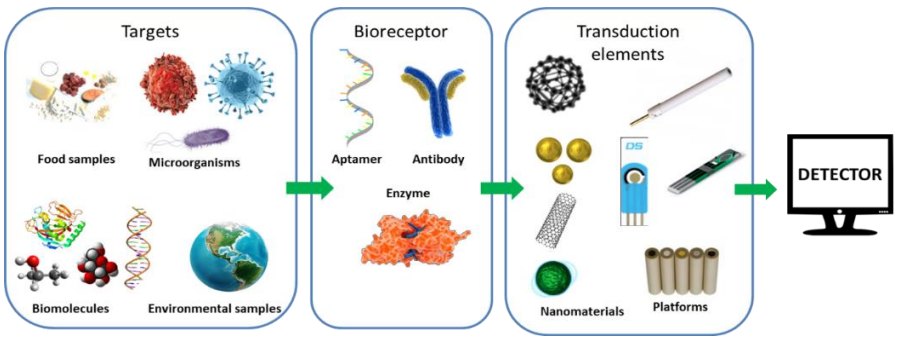


**Figure 2**. Schematic overview of approaches and components of aptasensor.

The supremacy of aptamers over antibodies makes aptamer technology more popular, giving rise to various applications in many fields, especially in biomedical, food, and

environment sectors [6–8]. In food applications, it is used in ensuring food safety by fast and effective detection of any kind of containments, pathogens, and allergens. This quick detection is essential as there is an increase in intentional or unintentional contamination in food production, even though the standard protocol is being followed. It is hoped that aptamers will prove to be useful in developing effective aptasensors with improved performance. This review is staged based on the various classes of nanomaterials exploited in the aptasensor fabrication and based on the different transduction systems utilized for analyte detection.

## 2. Integration of Nanomaterials into Biosensing Platforms

In various micro detection techniques such as electrochemical, colorimetry, luminescence, fluorescence, etc., nanomaterials have proven remarkable by giving high throughput performance. Their nano-scale size and high surface area facilitate aptamers to fabricate miniaturized equipment that requires low sample volumes, shorter assay time, and minimum operating costs. Moreover, the unique physiochemical properties combined with biocompatibility results in higher specificity and stability, thus adequately improving performance by efficiently accelerating electron transfers between electrodes and reducing analysis time [9].

Presently, a diverse range of available compounds falls under the designation of nanomaterials reviewed in various fields of applications in the past years [10]. Each plays individual constructive roles in nano-biosensing applications. Common few will be discussed by dividing them into several subcategories, as displayed in figure 3.

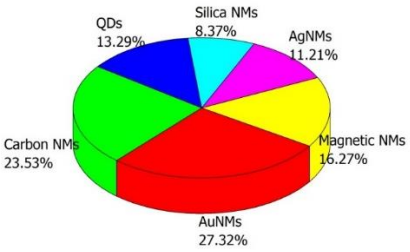


**Figure 3.** Classification of aptasensor based on nanomaterials utilized (data obtained from science direct).

### *2.1. Carbon nanomaterials.*

The detection of macro-biomolecule, such as protein biomarkers using carbon nanomaterial, is currently a rapidly growing field of interest, particularly in electrochemical studies. This was supported in parallel with bioconjugated aptamers with nanomaterials via terminal end functionalization to improve the interfacial adsorption properties, better electrocatalytic activity, high biocompatibility, and steady electron transfer kinetics compared to the traditional sensor materials, and many more. Herein, we will discuss a range of recognition elements assimilated into the biological sensors and how it is employed as a signal-transducing element.

#### 2.1.1. Carbon nanotubes (CNT), SWCNT & MWCNT.

Carbon Nanotubes (CNT) is made of hollowed-core cylindrical sheets of graphene that can be produced in many forms ranging from single-walled (SWCNT) to multi-walled carbon nanotubes (MWCNT) with varying thickness, layers, and conductive properties. CNT increases the surface area for the attachment of antibodies and aptamers while providing a

direct link between the sensor's surface and the recognition molecules. Besides the excellent adsorptive ability, chemical inertness, and high surface-to-volume ratio, the utilization of CNTs increases the overall specificity. It speeds up the electron transportation on the sensor [11].

To ensure a proper, intact and balanced distribution of CNT in solvents, various chemical and physical methods are employed to disperse the nanomaterial. For instance, ultrasonic processing, oxidative acids treatment, adsorption by non-polar organic solvents, the addition of surfactants, surface functionalization, composite mixtures are the methods that have been used for incorporating CNT in biosensors [12]. Out of all the mentioned techniques, the chemical approach is widely implemented. It reduces the destruction of the π-π system of $sp^2$ hybridization of carbon atoms in CNT [13].

SWCNT, being involved in many aptamer modifications, has been used in combination with other materials in electrochemical aptasensors to generate a significant difference in peak current. Rostamabadi *et al*. [14]used the nanocomposite of GO and SWCNT to modify the electrode surface. Later this was electrochemically reduced to ErGO-SWCNT. SWCNT was proven to prevent the possible agglomeration of the ErGO. It also functioned as a connection between ErGO and HER 2 aptamer, achieving a low LOD of 50 fg $ml^{-1}$. Appaturis's group also used the nanocomposite but prepared the rGO-CNT composite hydrothermally. They utilized the fibrous structure of the CNT as an immobilization matrix, thereby mediating the electron flow. The amino-modified Salmonella aptamer was bound onto the rGO-CNT matrix and the bacteria. Due to rGO and CNT's synergistic effects, the aptasensor detected whole Salmonella Typhimurium cells without any pre-treatment or DNA extractions. It could detect as low as $10^1$ CFU $mL^{-1}$ with a wide linear range of $10^1$ until $10^8$ cfu $mL^{-1}$ [15]. On the other hand, MWCNT dispersed in chitosan yielded even immobilization of the aptamers. This is due to the presence of a large number of -$NH_2$ and -OH groups in chitosan, forming covalent phosphoramidate bonds between amino group ($NH_2$) of chitosan and phosphate group ($[PO_4]^{3-}$) of the aptamer sequence [16]. Jalalvand's work highlights the amplified sensitivity provided by the MWCNT due to the high surface-to-volume ratio. Dual detection of prostate-specific antigen (PSA) by EIS and DPV was observed for the proposed aptasensor, and with EIS it gave a range of 1–200 pg $mL^{-1}$ and a limit of detection (LOD) of 0.5 pg $mL^{-1}$. MWCNTs have also been proven to be effectively combined with other carbon nanomaterials such as graphene quantum dots (GQDs), leading to the development of a 3D network for the hybrid nanomaterial with exceptional stability, higher conductivity, and faster electron transfer kinetics through π-π stacking interaction because of their similar conjugated structures [17]. Samie and Arvand devised a nanocomposite consisting of GQDs, NiO, AuNFs, and f-MWCNTs to immobilize the P4-aptamer to detect progesterone from biological fluids. The low LOD of 1.86 pM corroborates that f-MWCNTs, together with the other nanomaterials, served as an excellent transduction platform [18].

2.1.2. Graphene-based nanomaterials.

Graphene-based materials have been comprehensively reviewed with an increasing number of papers published involving graphene surface modifications in producing novel biosensors[19–21]. This broadly utilized material is known to have comparable mechanical strengths and remarkable electrical conductivity properties as CNTs. Graphene itself is a type of two-dimensional carbon nanomaterial composed of a single-layer sheet of $sp^2$-hybridized carbon atoms. Naturally, the interaction of graphene and aptamer will occur through the π-π stacking interaction between the bases of nucleic acids of aptamers and graphene plane [22].

There are various procedures involved in functionalizing graphene and graphene derivatives as the graphitic structures are inert. Functionalization is vital for the immobilization of aptamers onto substrates. The desired modifications, for instance, are physical adsorption, electrostatic adsorption, and oxidation treatment for carbonyl and carboxyl functional groups [23]. In a chemical modification of graphene functionalization, potential alteration of graphitic structures may occur; hence additional reaction step of free radical addition is needed to reduce the impact on graphene electronic properties [24]. To date, graphitic materials including graphene oxides [25], reduced graphene oxides [26], graphene nanoplatelets [27], graphene nanoribbons [28], chemical vapor deposition (CVD) prepared graphene [29] have been reported in combination with other nanomaterials for the fabrication of electrochemical aptasensors.

Graphene oxide (GO) is a graphene derivative containing several hydroxyls, epoxy, carbonyl, and carboxyl groups that serve as potential anchoring sites for various bioanalytes. Due to these hydrophilic groups' presence, GO demonstrated good dispersion in water and easy functionalization onto solid surfaces, and the stated biocompatibility makes it an ideal substrate for the preparation of electrochemical biosensors [30]. Ahour and Ahsani reported a basic label-free electrochemical aptasensor only by dispersing GO on a solid surface and double-stranded aptamer for capturing thrombin analyte [31]. A similar study was reported using GO nanosheets for different analyte detection. The GO is anchored onto diazonium functionalized electrode via electrostatic attraction, hydrogen bonding, and epoxy ring-opening enabling π-π stacking between hexagonal cells of GO and DNA base, rings to facilitate the immobilization [32].

Depending on the electrode surface, GO nanosheets may be less conductive due to widely exposed oxygen-containing groups; hence it is typically reduced to rGO. A single rGO sheet can load hundreds of reporter DNA via negatively charged phosphate backbones [33]. Shimaa's group highlighted the importance of controlling GO nanosheets' size by performing a comparative study utilizing microcystin- LR (MC-LR) aptasensor. Aptasensors were constructed by drop-casting GO solution of different sizes ranging from 0.22μm to >100μm onto separate disposable electrical printed (DEP) electrodes (Figure 4). Physical adsorption and covalent interactions were employed to immobilize the MC-LR aptamer. In physisorption aptasensor, the variance in binding signal considerably increased with the increase in GO sheet size. However, in chemisorption aptasensor, the binding signal variance decreased with GO sheet size increase [34]. Graphene functionalized with a carboxyl group, COO-GR, may be used to provide support for the aptamer's active sites. Additionally, nanocomposite can be easily prepared due to better solubility of COO-GR in water [35]. Further enhancement of the transducing signal on the sensor platform can be done by modifying graphene materials with other nanoparticles as composites [33]. Nano-graphite, GO-like properties with π-rich structure have been considered a promising energy acceptor. It can quench many fluorescent materials, including organic dye, quantum dots, and other metal nanoclusters. It is more cost-effective and easily facilitates subsequent modification than GO [36,37].

*2.2. Mesoporous silica nanoparticles.*

Mesoporous silica nanoparticles (MSNs) exist in various types and can be produced in a range of sizes. Due to their tunable porous structure, these nanoparticles have been extensively used in biomedicine applications for drug carriers [38].

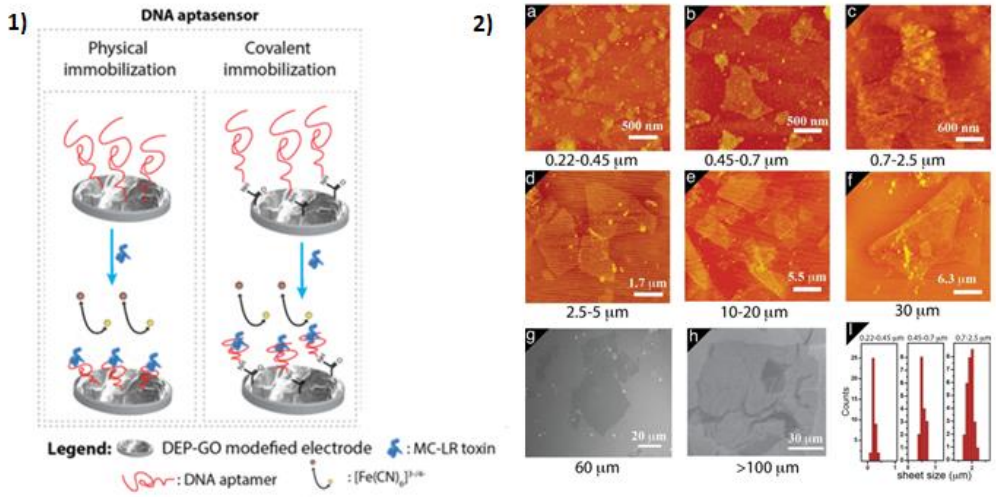


**Figure 4**. 1) Schematic representation of the aptasensor fabrication. 2) a–h Scan Asyst-mode atomic force microscopy (AFM)and scanning electron microscopy (SEM) images of different GO fractions and (I) Size distribution profile of three representative samples. [34] Copyright © 2020 Springer Nature.

Additionally, these mesoporous silica nanoparticles can adapt in great diversity, such as in surface functionalization mainly attributed to its large surface area. In Jamei *et al*. work, amino-MSN nanocomposite has been used in aptasensor platforms, whereby a stable aptamer immobilization was observed [39]. Eguilaz *et al*. utilized the combination of MWCNT and silica-carbon mesoporous nanoparticles to create a biosensor for the sensitive and selective quantification of nitrite ($NO_2^-$) and trichloroacetic acid (TCA) [40]. Meanwhile, Oroval *et al*. used MSN capped with single-stranded aptamer, which folds into a hairpin structure in the presence of arsenic metal [41]. Thereby the pores of MSNs were unblocked delivering the loaded fluorescent probe, Rhodamine B. Dehghani *et al*. utilized a double-stranded (ds DNA) capped MSNs instead of the hairpin aptamers, and this successfully capped the pores of the MSNs, leading to increased sensitivity of the fluorescent kanamycin aptasensor [42]. In the presence of kanamycin, the aptamer separates from the ds DNA structure leaving the MSN pores uncovered. This resulted in the release of Rhodamine B, increasing the fluorescence.

*2.3. Metallic nanomaterials.*

The assimilation of nanoporous metallic materials such as Au, Ag, Cu, Pd, Pt, in the form of mixtures of metals alloy in aptamers has shown promising potential in developing ultrasensitive biosensor detection. Biosensors fabricated using metallic nanomaterials proved to exhibit unique electronic structures, excellent signal amplification, great mechanical strength, and reported ease of biomolecules immobilization in matrices [43,44].

Different classification of interesting biosensor compounds has been developed to integrate metal oxides with aptamers, namely ZnO, $Zr_2O$, and $MnO_2$ [45,46]. Dual-mode detection using a combination of colorimetric and fluorometric was also reported as an added advantage of the metal nanoparticles recently [47].

2.3.1. Gold nanomaterials.

Gold nanoparticles generate distinct color changes depending on their size (up to 100 nm) because they possess unique localized surface plasmon resonance (LSPR) properties with high molar extinction coefficients. Thus gold nanoparticle aptasensors are suitable to be used

in techniques such as colorimetry, fluorometry, electrical and electrochemical, surface plasmon resonance, or surface-enhanced Raman scattering (SERS) [48]. This property has been exploited in most of the colorimetric assays [49] and SPR based sensors [50]. Colloidal AuNPs are used to generate color change, which can be observed by the naked eye resulting from sodium chloride (NaCl) solution. Studies state that aptamers could be non specifically adsorbed onto the AuNPs, even after binding with the target. Therefore, to improve the sensor's reliability, Alsager's group included centrifugation and resuspension steps after the vitamin D3 (VTD3) aptamer-target (vitamin D3) complex was formed. An immense rise of 4 fold was observed when compared with the original method, resulting in a low LOD of 1nM [51]. Taking advantage of the attractive and repulsive phenomena, Lee *et al*. proposed a simple colorimetric aptasensor to detect Bisphenol-A (BPA). The AuNP-aptamer complex dissociates in the presence of BPA due to the aptamer's high affinity towards the target. After that, the color changes from red to purple or blue in the presence of NaCl as a result of the aggregation of free AuNPs [52].

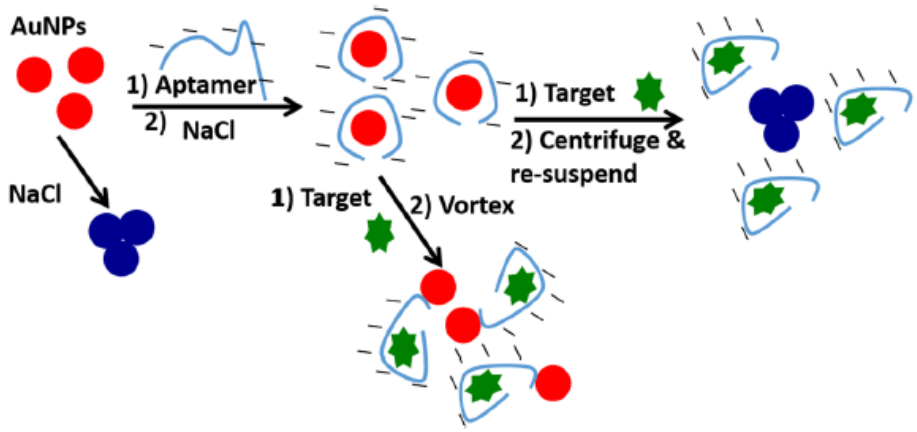


**Figure 5**. AuNP colorimetric aggregation-based sensing method. The Figure illustrates the proposed suppression effect of nonbinding flanking nucleotide on target binding signals, adhering to the particles after target detection, which prevents aggregation, and the role of the proposed method (centrifugation and resuspension) to eliminate the residual adhesion of these nonbinding sequences. [51] Copyright © 2018 Springer Nature.

Furthermore, gold functionalized on terminal aptamer is used as an amplification tool to enhance the measured signal [53]. Both the high conductivity and large surface area per unit mass improve the aptamer-based assay's overall sensitivity and selectivity. With the aid of stable gold nanomaterials, aptamer immobilization, layer-by-layer, and self-assembly are feasible through thiolate chemistry (S-Au bonds) onto sensor platforms [54]. In some cases, undesired self-aggregation and precipitation of gold particles may occur, therefore controlling the bioactivity reaction and protection of gold surfaces, pre-treatment with citrates, encapsulation using microemulsions, and dispersion in polymer matrixes may be used [55]. Table 1 summarizes some of the recent studies that used metal nano complexes to develop aptasensors.

2.3.2. Silver nanoparticles.

Similar to AuNPs, silver nanoparticles (AgNPs) also have exceptional extinction coefficients, making them the most preferred when designing fluorometric sensors [56].

Zhangs's group reported a fluorescent aptasensor with DNA silver nanoclusters (DNA-AgNCs) as a fluorescent nanomaterial. DNA-AgNCs are proven for their high quantum yield, strong photostability, low toxicity, and adjustable fluorescence emission [57]. AgNPs offer excellent properties in electrochemical sensing; it does not require harsh reagent for oxidation, ease of suspension and produces sharp peaks, thus improves the overall electrochemical performance. Reduced graphene oxide and silver nanocomposites (rGOAgNPs) were synthesized by Shi *et al*. to immobilize maximum aptamers and amplify the signal, and they obtained a low LOD of 0.3pM [58]. Considering the efficiency in catalyzing reactions of AgNPs, Cheng's group combined AgNps, luminol, and aptamer to fabricate an electrochemiluminescence based aptasensor to detect kanamycin. It was seen that AgNPs improved the luminol signal by stimulating the hydrogen peroxide catalysis [59].

*2.4. Magnetic nanoparticles.*

Magnetic nanoparticles (MNP) are often introduced in aptasensor to act as a ''separator'. MNPs can be functionalized with -OH, -COOH, and $NH_2$ functional groups, and hence further modification with conjugated aptamers can be achieved. In recent reports, MNPs are mostly based on superparamagnetic prevalently particles such as iron and iron oxide ($Fe_3O_4$) [60]. Depending on their geometry and compositions, these particles enable easy handling, modification, and manipulation of the assay. Moreover, MNPs provides an excellent interface for the electrocatalysis process of redox-active materials such as H2O2, O2, or NADH to prepare biosensors [61]. Other than its biocompatibility, these particles allow effective immobilization and hybridization of capture receptors, minimizing unspecific adsorption that can occur in complex samples and enable control towards the orientation of signal probes on the surface of disposable electrodes [62]. Thus, the signal's amplification can be accomplished by attracting the target analytes by an external magnetic field. Recently, He *et al*. reported a new approach for simultaneous detection of kanamycin (KANA), aflatoxin M1 (AFM1), and 17β-estradiol (E2) based on tripartite DNA structure-functionalized magnetic nanocomposites using microfluidic chip aptasensor [63].

*2.5. Quantum dots.*

Quantum dots (QDs) are zero-dimensional materials ranging from 1-10 nm. They are known to function as a reporter, and they offer many advantages over conventional fluorophores and dyes when they are employed as labels [80]. Typically, QDs are made up of compounds which are in a combination of Zn and Cd with Te and Se and also groups III-V and IV-VI [81]. QDs exhibit thermally and chemically stable properties, which provide good biocompatibility towards various detection and quantification application.

Due to the distinctive fluorescent properties, excellent photostability, high quantum yield, and high molar extinction coefficient, QDs are used as signaling tools to improve the limit of detection, sensitivity, and specificity in optical, fluorescence, and electrochemical methods.

QDs respond to the same excitation wavelength but emit at different wavelengths hence assisting multiplexing bioanalysis [82]. The multiplex detection has been successfully applied in Sun *et al*.'s work for recognizing two essential tumor markers, carcinoembryonic antigen (CEA) and prostate-specific antigen (PSA). Fluorescent mesoporous silica nanoparticles (MSN) were created by combining cadmium tellurium (CdTe) QDs and mesoporous silica nanoparticles (MSN), and later the aptamers were functionalized with this.

**Table 1.** Metal nanocomplex based aptasensors.

| Nanoparticle | Electrode | Aptamer modification | Role of nanomaterials | Target | Detection method | LOD | Reference |
|---|---|---|---|---|---|---|---|
| Silver nanoparticles (AgNPs) | Platinum electrode | 5' $NH_2$ | Immobilized aptamer through silver-amino bond | kanamycin (KAN) | ECL | 0.06ng $mL^{-1}$ | [59] |
| reduced graphene oxide-silver nanoparticles (rGO-AgNPs) + Prussian blue-gold nanoparticles (PB-AuNPs) | glassy carbon electrode | 5′-(SH) | large specific surface area catalyst for the redox reaction and assemble aptamers by Au-S bond | Acetamiprid (ACE) | CV | 0.30 pM | [58] |
| green nitrogen and sulfur co-doped carbon quantum dots (N,S-CQDs) + AgNPs | | Nil | N,S-CQDs as fluorescents and AgNPs as quenchers | adenosine (AD) | Fluorometry | 13 nM | [56] |
| silver nanoparticle decorated graphene oxide (AgNPs–GO) | Graphite screen-printed electrode (GSPE) | 3' $NH_2$ | As the redox probe | adenosine triphosphate (ATP) | DPV | 5.0nM | [64] |
| AgNPs | | Nil | As colorimetric elements | adenosine (AD) | colorimetric | 21 nM | [65] |
| gold@silver nanoparticles (Au@Ag NPs) | | 5′-Cy3 Cyanine-3 (Cy3) | SERS sensing platform | kanamycin (KANA) | SERS | 0.90 pg $mL^{-1}$ | [66] |
| gold nanoparticles (AuNps) | screen-printed carbon electrode (SPCE) | 5' $NH_2$ | Functions as the electrode modifier | cardiac troponin I (cTnI) | Amperometric | 1.0 pM | [64] |
| fern leaves-like gold nanostructure (FLGN) | Gold electrode | 5′-(SH) | Immobilization of aptamer through Au- SH bond | amyloid beta (Aβ) | DPV | 0.4 pg $mL^{-1}$ | [67] |
| Octahedral cuprous oxide – gold nanocomposite ($Cu_2O$-Au) | glassy carbon electrode | 5' $NH_2$ | electroactive toluidine blue (Tb) immobilized onto the Cu2O-Au through a stable Au-N bond | thrombin (TB) | Amperometric | 23fM | [64] |
| gold nanoparticles (AuNPs) + DNA-AuNPs-horseradish peroxidase (DNA-AuNPs-HRP) | glassy carbon electrode (GCE) | | large surface area as a nanocarrier for the enzyme HRP and DNA | aflatoxin B1 (AFB1) | DPV | $3.3\times10^{-4}$ ng $mL^{-1}$ | [68] |
| carbon nanohorns/gold nanoparticles (CNHs/AuNPs) | glassy carbon electrode (GCE) | 5′-(SH) | to assemble the aptamer by Au-S bond | carbendazim (CBZ) | EIS | 0.5 pg $mL^{-1}$ | [69] |
| gold hexacyanoferrate (AuHCF) + gold nanoparticles((AuNPs) | graphite screen-printed electrode (SPE) | 5′-(SH) | covalent immobilization of thiolated aptamers against | tumor necrosis factor alpha (TNF-α) | DPV | 5.5 pg.$mL^{-1}$ | [70] |
| gold nanoparticles (GNPs). | | | GNPs aggregation in high sodium chloride(NaCl) solution | Salmonella typhimurium | Colorimetric | 56 cfu $mL^{-1}$ | [71] |
| gold nanoparticles (AuNPs) | | 5′-biotin | ALP induced AuNP aggregation | ochratoxin A (OTA) | Colorimetric | 0.05 U·$L^{-1}$ | [72] |
| gold nanoparticles (AuNPs) | | | AuNPs aggregation under high-salt conditions | Chloramphenicol (CAP) and tetracycline (TET) | Colorimetric | 32.9 and 7.0 nM | [73] |
| gold nanoparticles (AuNPs) | | | poly(diallyl dimethyl ammonium chloride) (PDDA) induced AuNP aggregation | prostate specific antigen (PSA) | Colorimetric | 20 pg $ml^{-1}$ | [74] |
| reduced graphene oxide/molybdenum disulfide (rGO/$MoS_2$) composites and $Fe_3O_4$NPs | magnetic glassy carbon electrode (MGCE) | 5'-SH | $Fe_3O_4$NPs as both separator and as enzyme mimics | circulating tumor cells (CTCs) | DPV | 6 cells $mL^{-1}$ | [75] |
| gold nanoparticles (AuNPs) and magnetic nanoparticles (MNPs) | | | MNPs as separator and AuNPs to immobilize the aptamers | microcystin-LR (MC-LR) | surface enhanced Raman spectroscopy (SERS) | 0.002 ngmL $^{-1}$ | [76] |
| graphene oxide-ferroferric oxide (GO-$Fe_3O_4$) | SPCE | 5′-$NH_2$ | as aptamer carrier and magnetic catcher to capture the target | organophosphorus pesticides (OPs) | DPV | | [77] |
| $Fe_3O_4$ @$SiO_2$ | | | To immobilize the aptamer and to preconcentrate the target | Brucella melitensis | quartz crystal microbalance (QCM) | 100 CFU $mL^{-1}$ | [78] |
| magnetic nanoparticles (MNP) | GCE | 5′-$NH_2$ | as a separator to remove the unbound signal probes | Thrombin (TB) | SWV | 0.03 fM | [79] |

To eliminate signal cross-talk in the multiplex detection, CdTe QDs in different dimensions were synthesized to achieve two distinctive emitting wavelengths of 590 nm and 731nm [83].

At present, scientists are considering non-toxic QD compounds as an alternative to heavy metallic QDs that are less harmful to a biomolecule and its reaction and a threat to the environment [84]. Exceptional fluorescent properties were displayed by the colloidal nanoceria and graphene quantum dots (GQDs) synthesized by Tian's group. Fluorescence resonance energy transfer (FRET) from nanoceria to GQD was monitored in the presence and absence of the target ochratoxin A (OTA) to device the ratiometric fluorescent aptasensor [85]. Due to the high water solubility and low toxicity, CQDs/GQDs are being used as ECL coreactants also [85]. Luo *et al*. succeeded in designing a sensitive ECL aptasensor incorporating amine-functionalized $Ru(bpy)_3^{2+}$-doped silica nanoparticles ($NH_2$-Ru@$SiO_2$ NPs) and nitrogen-doped graphene quantum dots (NGQDs), whereby a low LOD of 1 fg $mL^{-1}$ of zearalenone (ZEN) was detected [86].

*2.6. Upconversion nanoparticles.*

Upconversion NPs (UCNPs) can emit visible luminescence under near-infrared (NIR) excitation that offers low auto-fluorescence background, good photochemical stability, large Stroke shifts, non-blinking and non-bleaching emission, tunable fluorescence wavelength, low toxicity, and ease of diffusion into biological samples [87]. Due to these advantageous properties, UCNPs have been regarded as more promising than traditional fluorescent materials like quantum dots, the down-conversion fluorescent materials used in fluorescence-based recognition. Additionally, the biological samples involved would not cause any unnecessary absorbance that induces auto-fluorescence and light scattering background during the process of UCNPs excitation through which NIR light was utilized in multi-photon absorption [88]. Inspired by these merits of UCNPs, Y. Wang *et al*. constructed an aptasensor to detect CEA using the FRET between UCNPs and graphene oxide (GO) [89]. The applications employing lanthanide-doped UCNPs have significantly increased as a result of their anti-Stokes emission. Established works have proven that UCNPs is the desirable choice as energy donors for luminescence resonance energy transfer (LRET) due to their no autofluorescence property [90]. Based on this, Wang's group developed a highly sensitive aptasensor to detect exosomes, utilizing the LRET that happened between donor rare-earth-doped upconversion nanoparticles (UCNPs) and acceptor tetramethylrhodamine (TAMRA) [91].

## 3. Approach/Method of Detection

Through the advancement of technology, diverse detection methods were also identified. Transduction methods can be mainly classified as electrochemical, optical, and mass-based. Herein we are discussing electrochemical and optical nano aptasensors in detail based on their modifications and sensitivity.

*3.1. Electrochemical.*

An ultimate electrochemical biosensor is generally characterized by having a high signal-to-noise ratio, femto- to pico-level detection with a broad linear range of analyte concentration. This type of sensor detection technique appears particularly promising for the fabrication of highly sensitive detection tools. A variety of configurations can be adapted to

facilitate aptamers as the recognition tools in gaining the optimal performance of bioreceptors. In this approach, the aptamer-based assay's overall purpose is to perform the quantitative analysis of an analyte in a sample with the highest possible sensitivity. Basic configurations may range from direct, sandwich, and competitive-type assays. The direct-type assay relies mostly on a single-site format in which a single aptamer is employed for the recognition process. Sandwich format assays engage double-site configurations that involve simultaneous binding of two oligonucleotides to reduce any binding interference. In contrast, competitive assays or displacement mode assay competes with labeled-target molecules or aptamers to limited binding sites [92].

Responses of signals are based on the change in current, voltage, potential difference, or impedance and can be generated by introducing catalytic labels, i.e., redox enzymes and nanoparticles towards electrodes [93]. A ratiometric approach was developed by Yang *et al*. employing the two different electroactive substances, ferrocene (Fc) and methylene blue (MB) to label the mucin 1 (MUC1) aptamers. Fc-Apt captured the MUC 1. The sandwich method, MB-Apt, was attached to the MUC 1, thereby generating stable MB signals [94].

Despite the ease of labeled sensing assay, most fabrication undergoes an expensive and tedious process of aptamer or target-labeling with a probe molecule, making them complicated. Additionally, non-specific adsorption and reduced affinity between the receptor and target may also occur, and hence researchers' preferred label-free detections [27,44]. In a label-free detection mechanism, non-labeled aptamers were used, where a simple electrochemical sensing technique could be explained by a change in conformation of different structures/patterns. When aptamer was immobilized on a conducting sensor, a primary signal would be generated, and after binding with its target molecule, a secondary signal could be measured. During the addition of the target molecule, DNA will be unfolded, causing the signal probe to attract or repel from the electrode surface leading to a signal enhancement or reduction, respectively. This is known as the ''signal on/'off' method. Integration of both signals on and off sense utilizing label and label-free strategies has been widely used in amplifying the detection of a target [95].

A range of electrochemical detection techniques in conjunction with aptasensors has been classified as amperometric, voltammetric, potentiometric, and impedimetric [96]. In amperometry, the oxidation/ reduction current generated at the electrode surface with the application of a constant external potential is measured. Many sensitive amperometric biosensors with low detection limits have been developed. MB served as the redox moiety in the amperometric aptasensors for saxitoxin (STX) [97] and ochratoxin A (OTA) [98].

The studies conducted based on voltammetry outnumbers all other electrochemical techniques. Unlike amperometry, voltammetry measures current under a range of potentials.

The main methods that contribute to voltammetry are cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), linear sweep voltammetry (LSV), and stripping voltammetry (SV) [99]. Reduction and oxidation of redox species are commonly analyzed by CV. In most studies, CV functions as the characterization method to study the layer-by-layer electrode modifications by reviewing the current signal change [100]. Like CV, LSV also measures the current when potential is altered with time. LSV is mostly conducted as a replacement for CV when only a forward scan is required [101]. Researchers widely use DPV and SWV as both approaches produce accurate results without the need for expensive materials and sophisticated procedures for sample preparation [102,103]. Nowadays, SV is extensively applied in the field of trace analysis. Metal NPs or

QDs work as the tracers where they are converted to specific cations in an acidic medium and later measured in the SV scan [104].

The potential of a solution between two electrodes is measured by potentiometry, and the solution remains unaffected during the process. Polymeric membrane ion-selective electrodes (ISEs) based potentiometry is a developed and widely applied analytical technology in physiological testing of key electrolytes. Potentiometric sensors are also employed to detect metal ions, small molecules, DNA, enzymes/proteins, bacteria cells, and toxins [104]. A "sandwich-type" potentiometric detection strategy was introduced to detect $Pb^{2+}$ ions by transforming the DNA strands into a stable G4 pattern [105]. The gold electrode had an increased concentration of negative charges as it was modified by the polyion oligonucleotide-labeled gold nanoparticles (AuNPs-DNA). The aptamer was immobilized onto gold dendritic (DenAu) three-dimensional nanostructures, which ensured high surface, optimum signal, and decrease in the potentiometric sensing platform's resistance.

Electrochemical impedance spectroscopy (EIS) measures the charge transfer resistance formed at the electrode surface. EIS has emerged as a promising tool for characterization to ensure that aptamer target binding has occurred on the electrode surface [106]. Bharti *et al*. demonstrated the electrode surface properties' changes during the fabrication of the mucin1 (MUC1) aptasensor through EIS. The charge transfer resistance increased with the binding of MUC1 aptamer as well as the target MUC1, proving the aptamer-target binding occurred [107].

#### 3.1.1. Electrogenerated chemiluminescence.

Electrogenerated chemiluminescence (ECL) involves the emission of light by species electrogenerated at the electrode's surface during the highly energetic electron-transfer reaction and excitation states. Generally, ECL biosensing detects the interactions between biological recognition elements and their corresponding target through emission changes by the electroactive species. The most common ECL electroactive species are conventional luminophores such as ruthenium complexes [108], luminol family [109] as well as quantum dots [110].

The initial reaction of ECL would involve a redox reaction at the electrode surface. During homogeneous chemical reactions, electroactive species would undergo an excitation state and emits light. There are a variety of strategies in ECL biosensing, which includes utilizing luminophore as a signal label, steric hindrance and impedance from biorecognition reaction (commonly used for sensing of biomacromolecules and cells), interaction between the analyte and ECL luminophore, the interaction of analyte and co-reactant, and finally ECL-resonance energy transfer (ECL-RET) [111]. Any inhibition of electrochemical redox reaction or annihilate radicals would promote a change of ECL emission, leading to the analyte's quantitative detection [81].

Among the ECL luminophores, extensive studies have been conducted on $Ru(bpy)_3^{2+}$ due to its high luminous efficiency [112]. $Ru(bpy)_3^{2+}$ based ECL mainly uses co-reactants. The most widely employed co-reactant is Tripropylamine (TPrA) [113], but it is proven to be toxic in real-life applications [114]. Therefore, researchers have developed alternative co-reactants that work efficiently in the $Ru(bpy)_3^{2+}$ system. The capability of black phosphorus quantum dots (BPQDs) modified with styrene-acrylamide (St-AAm) nanospheres (BSAN) to act as co-reactant to generate ECL with $Ru(bpy)_3^{2+}$ was demonstrated by Liu's group when they fabricated a sensitive lysozyme based aptasensor [115]. Khonsari *et al*. successfully designed an ECL aptasensor based on lysozyme's quenching effect on nitrogen-doped graphene

quantum dots – persulfate (NGQD-$S_2O_8^{2-}$) co-reactant system. AuNPs were also incorporated to achieve a higher sensitivity by immobilizing the SH-tagged aptamers onto an ITO electrode [116].

*3.2. Optical.*

Optical sensors consist of transducers that can capture signals in ultraviolet, visible, and infrared radiation from either a chemical, biological, or physical reaction. Correspondingly, electrochemical sensors are limited to heterogeneous assays because the aptamers, complementary oligonucleotides, or target molecules need to be in proximity to the electrodes' surface. The optical detection technique takes advantage of the conformational changes occurring in aptamers to produce or quench fluorescence particles. There are a variety of optical detection methods available, which include colorimetry, fluorescence, surface-enhanced Raman scattering (SERS), and surface plasmon resonance (SPR) [8]. This review only focuses on simple techniques that are suitable for miniaturizing biosensing platforms, i.e., colorimetric and fluorometric.

3.2.1. Colorimetric aptasensor.

The colorimetric detection method is based on a color change of assay in the presence of target molecules. Most researchers use the technique of assembly or disassembly of AuNP as the novel candidate of indicator for colorimetric assay. The impression of color change tends to be more favorable. It does not require any expensive and complex analytical instrument as the electrochemical detection sensors [49]. Depending on the analyte's concentration, different degrees of color change could be observed, reflecting on the absorbance value. The activity of color change could be measured directly using a UV-Vis spectrophotometer [117].

Smartphones, which are composed of built-in camera features with high resolution and light sensors, can be used to produce a point-of-care testing detection for bioanalysis application. Xiao *et al*. reported smartphones in a colorimetric application as a signal readout to detect mercury-based on aptamer nanosensor [118]. Due to its simplicity and effectiveness, the studies based on smartphones in colorimetric assays showed a surge. Liu *et al*. guaranteed rapid and cost-effective food safety inspection by detecting the presence of streptomycin in food samples using a 3D printed smartphone-based platform (SBP), where aptamer-conjugated AuNPs served as the colorimetric indicator and a removable optical unit connected to the smartphone for light detection and data processing [118]. A similar approach was employed to detect arsenic As (III) from contaminated soil [119], using a smartphone as the detector.

3.2.2. Fluorescence aptasensors.

In principle, fluorescence detection relies mainly on the excitation and emission effects of fluorophores. The extinction coefficient, quantum yield, wavelength, anisotropy, and energy transfer are all significant features that cause these fluorophores to behave as a signaling molecule and assist with aptamer-target binding.

A commonly adopted fluorescence design is an aptamer-based molecular beacon. Aptamer beacons may adopt two or more conformations, whereby one allows ligand binding. A quenching pair is usually required consisting of fluorophores and quenchers dye, which can be functionalized onto aptamer through covalent or non-covalent bonds by embedment, electrostatic and hydrophobic interactions [120]. A potential problem with quenching aptamer

beacons is that some other ligands or solvents may interfere with quenching leading to ‘‘false ’positive’ signal. Hence to avoid the problem, beacons can rely upon Fluorescence Resonance Energy Transfer assay (FRET). FRET is a rapid, ultrasensitive type of fluorescence detection that can be constructed based on the energy transfer between two fluorescence molecules, namely donor and acceptor [121].

It is known that the usage of organic dyes causes photo-bleaching in conventional FRET systems [122]. Therefore, photostable QDs are employed as fluorescent dyes because of their high-efficiency energy transfer, their electronic and optical properties. The binding of its cognate ligand onto the aptasensor should stabilize the native structure of aptamer, altering the distance between the fluorophore and quencher, causing an easily detectable change in fluorescence intensity [123]. CdTe, a typical QD was employed by Zhou *et al*. for the sandwich-structured detection of alpha-fetoprotein (AFP) [124] based on FRET between QDs-AuNPs conjugate pairs.

More comprehensive application of UCNPs in FRET-based biosensors is observed due to its improved signal-to-noise ratio by reducing the background luminescence. A sensitive FRET aptasensor was devised by labeling the aptamer with UCPNs (Apt-UCNPs) and later, this was adsorbed onto a GO surface. UCNPs fluorescence was quenched by GO through FRET and as the target diazonin was added, aptamer bound to diazonin, which resulted in desorption of UCNPs from GO. Therefore UCNPs fluorescence was regained [125].

Researchers are now integrating smartphones in biosensing systems due to its software's capability, and it is seen as an effective platform for on-site detection. Lee *et al*. utilized smartphones for imaging-based fluorescence microscopy on a microarray platform using a dual-wavelength fluorescent detection method to reduce false detection [126]. The result shows significant improvement compared to conventional detection, ELISA. Hence this approach provides an excellent potential for point-of-care detection of biomolecules. In another study, a lab-on-smartphone platform was established by utilizing fluorescent magnetic nanoparticles (FMNPs). Besides being the on-chip capturer, FMNPs also quantitatively detected *Staphylococcus aureus* cells (*S. aureus*) with smartphone imaging in a limited time of 10 minutes [127].

## 4. Conclusion

The availability of umpteen articles on nanoaptasensors undoubtedly proves that the incorporation of nanomaterials enhances the biosensor properties in several aspects. This review classified the nanoaptasensors based on the nanomaterials used and the method of detection (electrochemical and optical). Same nanomaterials are recognized for the different properties, and this facilitates the utilization of them in numerous detection methods—for example, gold nanoparticles and graphene-based nanoparticles and widely utilized in electrochemical and optical-based sensors.

In most biosensors, various nanomaterials have been coupled to generate signal amplification by bringing about a highly efficient target binding and recognition. However, it is often suggested that an ideal aptasensor design is constructed when the labels on aptamers are eliminated. The labeling is usually expensive, complicated, and is considered a cumbersome process. Researchers have been trying to overcome these disadvantages of labeling by including in situ redox molecules during the aptasensor fabrication.

Besides the many benefits that aptamer possess as a sensor substrate, there are still a few hurdles that need to be addressed. The challenges include the stability of aptasensors for

sustainable performance, reusability of sensors, sensitivity and selectivity in a complex real sample, accuracy, cost-effectiveness, and portability of detection methods, simplicity in fabrication steps rapid attainment of results. In the future, the parameters of folding conditions like the buffer components, the temperature required etc., need to be carefully evaluated, as these factors indirectly contribute to the sensitivity and stability of the aptasensor. Although, in the development of aptamer-based sensors, detection devices are mainstream, yet it requires significant improvement in optimization for consistency and reproducibility.

Even though countless aptasensors have been reported, there is still a lack of multiplexed analysis. The multiple target detection will enhance the sensor's sensitivity and reliability, especially when detecting serum-based biomarkers, allergens, or contaminants. Miniaturization is of the utmost importance when developing POC analysis systems. Therefore, screen-printed electrodes or microchips modified with nanomaterials should be extensively used in sensor fabrication. Continuous effort in the development of biosensors is needed to improve immobilization and sensing strategies of aptamers on transducer surfaces. It can be hoped that future studies would analyze the problems stated so that a cost-effective and user-friendly nanoaptasensor could be successfully developed.

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## Conflicts of Interest

The authors declare no conflict of interest.

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