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REVIEW ARTICLE

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# Carbon Dots in the Detection of Pathogenic Bacteria and Viruses

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**ABSTRACT**

Bacterial and viruses pathogens are a significant hazard to human safety and health. In the imaging and detection of pathogenic microorganisms, the application of fluorescent nanoparticles is very useful. Carbon dots and quantum dots are preferred in this regard as labels, amplifiers, and/or electrode modifiers because of their outstanding features. However, precise diagnostics to identify numerous harmful bacteria simultaneously still face considerable hurdles, yet it is an inevitable issue. With the growing development of biosensors, nanoproduct-based bio-sensing has recently become one of the most promising methods for accurately identifying and quantifying various pathogens at low cost, high sensitivity, and selectivity, with time savings. The most recent applications of carbon dots in optical and electrochemical-based sensors are discussed in this review, along with some examples of pathogen sensors.

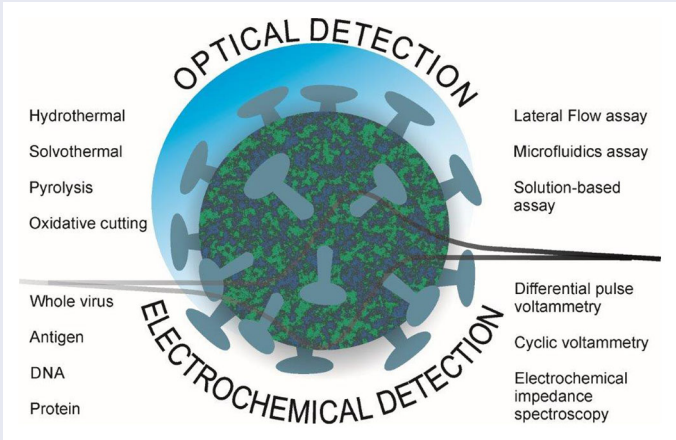
**KEYWORDS**

Pathogen; carbon dots; graphene quantum dots; virus detection; bacteria detection; optical sensor; electrochemical sensor

**HIGHLIGHTS**

- Simultaneous and early detection of pathogens is a critical issue in the management of readily spread to prevent epidemics.
- Carbon dots-based biosensors are more preferred in detection of pathogens due to high selectivity and sensitivity, as well as quick and cheap point-of-care platform.
- Summary of recent advances in the design of optical and electrochemical biosensors for the detection of pathogens.

**GRAPHICAL ABSTRACT**




## Introduction

Pathogenic viral infections have recently posed a significant threat to global health, particularly for the coronavirus 2019 (COVID-19). Consumption of polluted water and/or food products infected with viruses, bacteria, parasites, or chemicals that cause foodborne illnesses is one of the most severe threats to public health and a significant impediment to socio-economic growth worldwide. The development of a quick, selective, and accurate technique of pathogen detection is a critical issue in the management of readily spread illnesses. The primary impediment to advancement in this required field is the lack of real-time detection techniques. The most sophisticated detection method for infectious diseases is molecular diagnostics, which uses DNA and RNA biomarkers. Pathogenic infection identification and detection approaches include polymerase chain reaction (PCR),

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traditional antibody-based techniques such as enzyme-linked immunosorbent assay (ELISA), and conventional plate counts. Although these techniques offer high specificity and sensitivity, they are labor-intensive and time-intensive due to extensive sample preparation. In addition, they need somewhat expensive equipment with trained personnel to perform them.[1] As a result, quick and cheap identification techniques with excellent sensitivity and selectivity are still required. New innovative approaches are being developed to provide portable, fast, accurate, and affordable point-of-care platforms, such as the application of nanotechnology and current improvements in biosensor research.

This review covers the synthesis, mechanism, and use of CDs, particularly in the surveillance and control of harmful bacteria and viruses. To the best of our knowledge, there is no review paper in the area of virus and bacteria detection. Therefore, there is a need to offer an overview from many viewpoints (for example, CDs application in pathogens detection), review latest works, and address future work challenges. We intended to present a review to evaluate and classify recent approaches in order to help future researchers. We present an overview of the current developments in pathogen detection biosensing through several new techniques and methodologies based on CDs and GQD usage. Finally, the challenges associated with CDs in pathogen monitoring and future prospects are discussed.

## Advances in detection of pathogenic bacteria and viruses

Humankind has fought many different forms of bacteria and viruses throughout the years, which are the source of most infectious illnesses in humans, animals, and plants, resulting in financial, health, and life losses.[2,3] To show present or previous exposure to an infectious illness, it is critical to identify the presence or absence of the infectious agent or its matching antibodies in clinical testing.[4] Therefore, accurate and fast diagnosis of the causative pathogens is crucial to applying the appropriate treatment, controlling transmission, minimizing unnecessary consumption of antibiotics, and saving people's lives.[5]

Different traditional diagnostic procedures have been developed and applied to detect and identify harmful bacteria and viruses.[6] The systems include virus culture, direct and indirect immunofluorescence tests, and polymerase chain reactions (PCR),[5] and the most common pathogenic bacterial diagnosis detection methods rely primarily on DNA-based PCR and mass spectrometry to carry out phenotypic identification in the chromogenic medium.[7] In these systems, the genetic material is detected by nucleic acid testing. Generally, after collection the sample, copies of genetic material are produced through a series of chemical reactions.[8] Real-time polymerase chain reaction (RT-PCR) has been used as one of the gold standards for bacteria and virus detection. This procedure, however, needs 3–4 h of time. More importantly, the method based on immunoreaction can detect a single pathogen and challenge to detect multiple pathogenic species simultaneously. In terms of multiplex detection, multiple polymerase chain reaction assay (mPCR) seems to be the effective simultaneous detection of numerous species in practical applications.

In order to specifically amplify DNA or RNA generated from several target bacteria or virus, the concept is to put various primers into a single identical PCR apparatus to detect several target bacteria or viruses at once. To date, the mPCR-based testing could certainly also be regarded as one of the unique PCR technologies but usually requires complicated pretreatments and decoding analysis. Therefore, the development of alternative technologies for simultaneous detection of numerous pathogens to preserve public and environmental safety is of considerable importance.[7] For a conventional reason, most immunology and microbiology laboratories include antibody-based testing tools used to detect infectious or clinical pathogens.[9] These technologies are considered to be the most systemically sensitive and reliable approaches for pathogenic microorganisms detection to date. The complex pretreatments and decoding analytical processes make them time-consuming, labor-intensive,[7] sophisticated and expensive.[6] As a more straightforward method, the reverse transcription loop-mediated isothermal amplification (RT-LAMP) can detect a few copies of target sequences under isothermal conditions within one-hour, shorter than the RT-PCR, via a reverse transcription isothermal amplifier. However, false-negative nucleic acid test results have been reported due to a variety of variables, including patient sample quality, viral load and distribution, and the uniformity of the detection method.[10,11]

Innovative nano-based structures and nanobiotechnology are the focus of trends in fast, sensitive, and reliable pathogenic species detection to control and prevent/reduce their propagation.[3] These methods are usually based on molecular diagnostics, which have the ability to identify the presence of a disease by detecting its genetic material or the infection's distinctive markers.[3] Furthermore, sensors have been primarily developed, with a focus on miniaturization through the use of novel materials for sensor fabrication, as well as the intriguing properties of nanomaterials, which have a small size and a large surface area giving them tremendous potential for a wide range of applications. Nanomaterials for the quick and sensitive detection of harmful bacteria and viruses and the miniaturization of sensing instruments have recently been adopted as flexible signal translation and amplification tools.[6] In particular, due to its simplicity, features, and economic efficacy, the naked eye visual identification of targeted species has received great attention. Antigen and antibody testing have also been carried out extensively in recent years as an exciting method to discover the infection. Significant kinds of antigen testing include lateral flow assay (LFA) and enzyme-linked immunosorbent assay (ELISA) tests.

ELISA is a laboratory-based test, which requires experienced experts and which takes 1–3 h to complete. Up to 96 samples can be examined by per batch using this approach.[8] Antibody testing is helpful in detecting previous or advanced illnesses. On the other hand, the development of microfluidic and immunoassay technology based on

paper, plastics, and polymers has become more attractive to biomedical research organizations. It might provide an alternative to a lower-cost diagnosis of viral illnesses. LFA is the usually recognized cost-effective antibody test that requires no specialist immunoassay equipment. It is a reasonably quick test procedure, resulted in 15–20 min. New aptamer-based technologies have pushed the shift from antibody to aptamer-based testing because of the same specificity, increased susceptibility, low production costs, and flexible adjustment to the multi-faceted, high throughput fashion of chemiluminescent, electrochemical, or fluorescent detection as compared to antibody-based testing by aptamer-based assays.[9] Another recent pattern is the visible and faster alternative to standard procedures, including the RT-PCR, with clustered regularly interspaced short palindromic repeats (CRISPR). The invention of fast (<40 min), easily implemented, and exact CRISPR–Cas12-based LFA for respiratory swab RNA extract detection has been reported by Broughton et al.[12] The assay uses simultaneous, reverse transcription and isothermal amplification using loop-mediated amplification (RT–LAMP) for RNA extracted from nasopharyngeal or oropharyngeal swabs in universal transport medium (UTM), followed by Cas12 detection of predefined coronavirus sequences, after which cleavage of a reporter molecule confirms detection of the virus. Another method for detection of viruses and bacteria is based on molecularly imprinted polymers (MIPs), and surface-imprinted polymers (SIPs). Tremendous progress has been achieved, but numerous obstacles persist compared to antibody-based systems, such as sensitivity and selectivity. Nanopatterned electrodes are expected to enhance the design and use of MIPs/SIPs to produce better, more efficient cavities with a great spatial corresponding effect.[13]

The approaches above are the recent successful advances in the detection of pathogenic bacteria and viruses; however, the future development is expected to focus on the sensitive and portable assay with advancements in the fabrication of (nano)materials, point-of-care testing outside of the clinical diagnostic laboratory, such as clinics, local emergency rooms, airports, and other sites.

## Materials for detection of pathogenic bacteria and viruses

Worldwide health and food security are constantly faced with human and plant diseases induced by bacteria, viruses, and other pathogens. Pandemics such as Ebola, SARS, MERS, Swine Flu, and COVID-19 (ongoing) have caused huge losses worldwide. For instance, the COVID-19 virus has caused hundreds of thousands of deaths and has also had a massive effect on the world economy. Therefore, rapid diagnosis plays an increasingly critical role in controlling and preventing these diseases.[14–16] Pathogens are disease-causing microorganisms, and their existence should be observed accurately. However, traditional detection methods have several disadvantages, such as being time-consuming, complex, and labor intensive. To overcome such weaknesses, nanomaterial-based biosensors have attracted the attention of researchers as alternative candidates to enable portable, fast, easy, and direct diagnosis of target pathogens on-site.[17–19] In this section, nanomaterials fabricated for the assay of pathogens are listed and discussed.

Fluorescence-based tests using organic dye molecules are involved in the assay of pathogenic bacteria and viruses. Optical gas sensors are generally produced from two types of organic dyes, metalloporphyrins and metallo-phthalocyanines. These organic dyes have markedly expanded electron systems and exhibit stability at room temperature. These dyes, phthalocyanines were discovered as excellent sensing materials for optical gas sensors based on changes in optical absorption of amorphous thin films. Although these dyes provide a sensitive, safe, and cost-effective detection system, they have several disadvantages. Organic dyes are sensitive to photobleaching and are often not bright enough to quantify a particular signal on the background. In addition, the fluorescence spectra of organic dyes are not symmetrical, and each fluorophore is characterized by its specific optimal excitation wavelength, limiting its multiplexing capabilities. Nanomaterials produced with current developments in nanotechnology have several advantages over conventional dyes.[20–23]

The microparticle amplified assay can make specific detection of target bacteria. In addition, the use of microparticles leads to a susceptible and specific sensor for bacterial identification and quantification.[24,25]

Nanotechnology is one of the fields that attracts and develops the attention of researchers in many fields such as medicine, agriculture, and engineering. Nanomaterials, typically in the range of 1–100 nm in size, are excellent candidates for the detection of pathogens, thanks to their improved surface-to-volume ratio and unique chemical, optical, and electrical properties.[26–31]

### *Nanomaterials used in pathogenic bacteria and virus detection*

Many techniques and methods have been proposed for the assay of pathogenic bacteria. However, thanks to the properties of nanomaterials, fast, highly sensitive, and special devices can be developed for accurate bacteria analysis. As a result, nanobiosensors take advantage of the chemical, electrical, optical, and magnetic properties of nanomaterials to detect pathogens.[26,30,32–35]

Viruses that are 20–900 nm in size are particulate and exist in different morphological forms. Today, thanks to the unique properties of nanomaterials (physical, chemical, mechanical, magnetic, etc.), they are used effectively in virus detection. Various nanomaterials, including quantum dots, graphene, carbon nanotubes, gold nanoparticles, silver nanoparticles, and magnetic nanoparticles, are utilized as nanosensors to detect pathogenic viruses.[16,17,27,36–38] Some examples of nanoparticles used in pathogenic bacteria and virus detection are explained below.

### Quantum dots (QDs)

QDs are promising in detecting pathogenic bacteria because of their excellent physical and chemical (especially optical) properties. QDs are valuable materials for the rapid and precise detection of pathogenic viruses to facilitate early diagnosis and monitoring of viral diseases. Detailed information is provided in other parts of the review.

### Graphene

Graphene-based biosensors have been extensively developed in recent years, thanks to graphene's small size, unique properties, and good biocompatibility.[27,39] One of the processes to increase biosensor sensitivity is the use of materials with large surface area and conductivity.[40] Graphene is one of the materials used as an electrode modifier. The biosensor combined with graphene can improve the performance of the sensor in terms of sensitivity, selectivity and repeatability. Graphene obtained by graphene oxide (GO) reduction often has functional groups that are advantageous for electrochemical approaches.[41] GO containing polyfunctional groups such as carboxy, epoxy, ketone, and hydroxy groups is very suitable for improving its properties and expanding its scope of application.[42]

### Carbon nanotubes (CNTs)

The superior physicochemical properties of carbon-based nanomaterials enable these materials to be used as a component in biosensors. In particular, the high electrical conductivity and enhanced surface area of carbon-based nanomaterials increase the sensitivity of the sensors.[43] CNT-based biosensors have high sensitivity and selectivity thanks to their high surface areas.[36]

### Metal and metal oxide nanoparticles

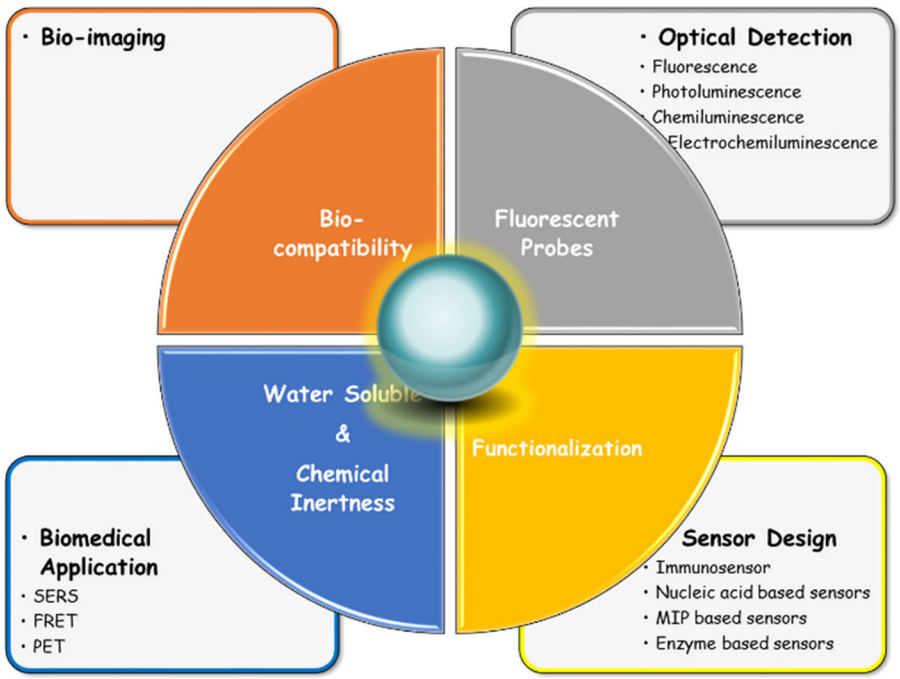
*Silver nanoparticles (AgNPs)*: AgNPs are one of the most commonly used nanomaterials due to their antimicrobial properties.[44] Thanks to the fluorescent properties of AgNPs, high-precision optical-based biosensors are designed and used for the detection of pathogen bacteria and viruses.[45]

*Magnetic nanoparticles (MNPs)*: MNPs provide a variety of easy and inexpensive methods for synthesis and allow the reacted target molecules to be easily separated from unreacted molecules.[46–48]

*Gold nanoparticles (AuNPs)*: Apart from the existing techniques that are widely applied in the detection of pathogenic bacteria and viruses, several new approaches based on nanoparticles have been developed.[38] AuNPs are known to be suitable for numerous biosensing functions and applications.[49] AuNPs can be linked by thiols, disulfides, and amino groups.[50] These features are used to design different biosensor types used in colorimetry analysis, luminescence analysis, surface plasmon resonance, and fluorescence analysis.[51]Its unique photonic, electrical and catalytic properties, combined with the molecular interaction specificity of various biomolecules, enable very sensitive, and rapid detection.[52]

## Carbon quantum dots

High-dimensional materials can be made more efficient and multi-functional by reducing their size to nano and converting their structures to zero-dimensional size. Zero-dimensional (0 D) nanomaterials with mostly spherical or hemispherical structure have a diameter of less than 100 nm.[53] One carbon family, zero-dimensional carbon quantum dots (CDs), have their extraordinary properties such as inexpensive, ecological-friendly, large surface area,




**Scheme 1.** Scheme of multi-functional properties of CDs

high electrical behavior, and easily bonded surface functional groups.[54] On the other hand, tedious and harsh purification processes, poor control over size, multi-step process, and high consumption of energy-consuming when synthesized can be referred to as disadvantages of CDs. Xu et al. reported that CDs' first isolation and characterization was performed in 2004.[55] Carbon quantum dots with dimensions of <10 nm are hemispherical fluorescent particles.[53] CDs with physicochemical properties similar to carbon-structured nanomaterials have excellent properties in luminescence, which are up-converted to photoluminescence and chemiluminescence. Carbon dots (CDs) are becoming quite well-known for their multi-functional properties for the concurrent and multiple detections of biomolecules.[56]

CDs have various properties such as photoluminescence, biocompatibility, ease of functionalization, water solubility and chemical inertness, which result in their use in bioimaging, optical detection, biomedical application, and sensor design.

## Synthesis methods of carbon quantum dots

Quantum Dots are synthesized with two different main techniques; "top-down" and "bottom-up." QDs are prepared through physical, chemical, or electrochemical methods.[57] The bottom-up synthesis of CDs proceeds through the carbonization of small molecular precursors. In particular, one of the most widespread bottom-up synthesis routes produces CDs from a mixture of citric acid and a nitrogen-containing molecule such as urea.[58] Concerning the top-down approaches, the precursors are extended carbon structures or nanostructures, such as graphite, amorphous carbon, carbon fibers, nanotubes, and fullerene, which are fragmented chemically or physically in order to obtain very small carbon nanoparticles.[59] Top-down synthetic routes are generally much more complex and time-consuming, and very often yield CDs with lower emission quantum yields.[60] However, it has been argued that they allow for much better structural control and purity of the end-products.

The top-down approach:

- Arc-discharge method,
- Laser ablation method,
- Electrochemical oxidation,

The bottom-up approach:

- Thermal decomposition,
- Microwave-assisted method,
- Hydro/Solvothermal method,
- Template method.[61]

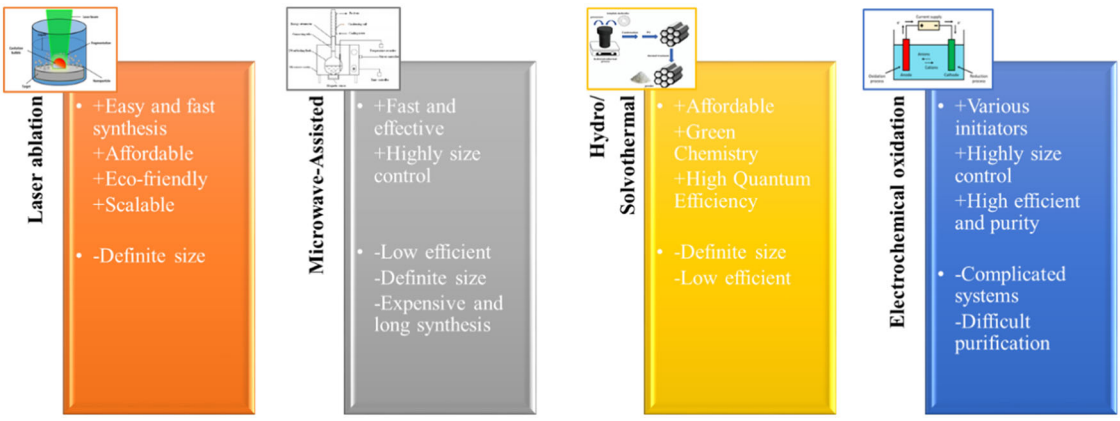
The advantages and disadvantages of the main synthesis processes of CDs are shown in Scheme 2.

### Hydro/solvothermal methods

One of the widely used techniques is hydrothermal carbonization(HTC) for the easy synthesis of CDs. In this technique, some glucose derivatives, some fruit juices, chitosan, and citric acid are used as precursors. Therefore, this method has become cheaper, eco-friendly. Nevertheless, it is complicated to control the quantum dots being synthesized by this method. Solvothermal method is a one-pot method of producing chemical compounds. Solvothermal and hydrothermal methods are performed in astainless-steel autoclave. While an aqueous solution is used in the hydrothermal method, a non-aqueous solution is used in the solvothermal method. The polarity of a non-aqueous solution affects the optical properties of CDs in carbonization process. Moreover, the optical properties of CDs are affected by surface coating and functionalizing, N-doping, and route of synthesis. Many different sizes of quantum dots are synthesized, changing the reaction temperature, process time, solvent solution, surfactant, and precursor. The waste materials are used in the hydrothermal process for the synthesis of novel nanomaterials. Therefore, it contributes to the green environment. Gan et al. reported that CDs are synthesized using waste tea leaves and peanut shells by a one-step hydrothermal method. The eco-friendly method gives an important opportunity to sustainable sources.[62]

### Laser ablation

Another comprehensive synthetic technique of carbon-derived nanomaterials is laser ablation. Laser is used as an energy source to ablate solid target materials. In this process, the energy source is intensified at a specific area on a solid surface to evaporate the material. Its disadvantages include




Scheme 2. The advantages and disadvantages of the main synthesis processes of CDs

the amount of energy lost by scattering and absorption and the inhomogeneous distribution on the surface of the samples. On the other hand, CDs with a smaller size would be fabricated thanks to a continuous flow jet, and this strategy reduces unnecessary energy consumption.[63]

### Microwave-assisted method

As an alternative synthetic technique, the microwave-assisted method is quite simple, fast, and selective with homogeneous heating. This technique has a large-scale application area. The microwave-assisted process allows fast production of large quantities. CDs could easily interact with microwave radiation, which causes a rapid heating rate and localized heating. On the other hand, the small penetration depth of microwave radiation in some substances is one of the limitations of this method.[64]

### Electrochemical oxidation

The electrochemical approach is one of the widely used methods to synthesize CDs by utilizing different bulk carbon materials. Carbon fiber, graphene, and graphite are used as precursors. Size control of CQD is difficult in traditional electrochemical synthesis. Filtration or chromatography facilitates minimizing the size distribution of CDs.

### Arc-discharge method

CDs have had significant attention since the discovery of carbon nanotubes synthesized with the arc-discharge method. CDs are used instead of heavy-metal semiconductor quantum dots because of their multiphoton fluorescence, tailored surface modification, and biocompatible properties. CDs have been considered promising candidates in solar cells, light-emitting devices, and bio-imaging.[65]

### Thermal method

In ordinary thermal decomposition, a substance or compound decomposes chemically by the action of heat. Thermal decomposition reactions are generally endothermic. This type of decomposition reactions is either irreversible (decomposition of starch, proteins) or reversible (decomposition of ammonium chloride, limestone). This method offers various advantages, such as easy to operate, less time consuming, low cost, and large-scale production.

### Template method

This approach has also been utilized for the preparation of nanosized carbon dots. This approach contains two stages:

i. developing carbon dots through calcination in the proper mesoporous template or silicon spheres
ii. Etching for removal of the support and generation of the nanosized carbon dots

### Application of carbon quantum dots

Zero-dimensional nanomaterials are of great importance in biomedical applications. For example, in biosensors and biochips, CDs can be used to develop new generation of biosensors with low energy consumption due to their biocompatible and tailored-surface modification.[53] Moreover, CDs have a very important place in bio-imaging, bio-sensing, and target delivery. Fluorescent CQDs are superior candidates due to their low toxicity and physicochemical properties different from other heavy metal-semiconductor quantum dots. Biosensing, analyte detection, and monitoring, wherein a key feature is coupled molecular recognition and signal transduction, is one such field that is significantly advanced by the use of CDs.

Five main surface modification techniques, including doping, surface functionalization, polymer capping, nano-composite, and core-shell structures, are applied to obtain the best biosensor.[66] The various functional groups (–OH, $–NH_2$, etc.) on the surface of CDs offer effective anchoring sites and active sites for the fabrication of high-performance platforms.

The effect of heteroatom (N, S, P, B, etc.) in the CDs shows the charge distribution and enhances the electron transfer via internal interactions. The doping or surface functionalization can enhance the topical chemical, optical properties, surface reaction activity, and biocompatibility of CDs. CDs as electrode surface modifiers, alone or in connection with polymers, molecular imprinting technology, carbon-based nanomaterials and metal nanoparticles, are reported for electrochemical sensing of different types of analytes. Therefore, the sensitivity of biosensors is increased by modification with CDs.[67] Moreover, CDs are increasingly used as advanced labels for signal amplification and they contribute to the immobilization of bioreceptors in label-free electrochemical biosensing of biomarkers.[68]

It is expected that the developed biosensors are applied to biological matrices for the determination of analytes such as metal ions, acids, proteins, thiols, polypeptides, DNA and miRNA, water pollutants, hematin, drugs, vitamins, and other chemicals. Generally, the aim is to reach nanomolar and even lower concentrations. CDs modified biosensors provide this purpose with different mechanisms, including fluorescence resonance energy transfer (FRET), fluorescence quenching, photo-induced electron transfer (PET), static quenching, dynamic quenching, inner filter effect (IFE), and energy transfer.[69]

## Carbon quantum dots-based pathogenic bacteria detection

Pathogenic bacteria found in food are the primary cause of many harmful health infections across the world. *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter jejuni*, *Salmonella* spp., *Bacillus cereus*, *Escherichia coli*, *Vibrio* spp., *Shigella* spp., *Listeria monocytogenes*, and *Legionella* spp. are the most common pathogenic bacteria that cause outbreaks across the world. Most pathogenic bacteria have an infectious count of less than 10 colony-forming units (CFU). Traditional microbial culture-

based assays for sensing pathogen bacteria using differential culture mediums take more time because of multiple processes such as pre-enriched, enriched, selective screening, and validation. The interoperability of the results of these steps is also problematic. Another disadvantage is their lack of sensitivity. So, to identify and treat pathogen illnesses, it is extremely desirable to design low-cost, selective, sensitive, robust, and real-time detection and quantification technologies for pathogen microorganisms. Immunological assays, such as immunochromatographic strip assays (ICS), chemiluminescent immunoassays (CLIAs), and enzyme-linked immunosorbent assays (ELISAs), are interested in real-time monitoring of pathogens in a short time in comparison with traditional methods. However, they have some drawbacks, such as the poor affinity of numerous antibodies to their target, cross-reactivity with closely related antigens, and low sensitivity for pathogens with low abundance. Nucleic acid-based diagnostics using polymerase chain reaction (PCR) provide numerous benefits, including detecting smaller doses of target nucleic acid, faster detection time, and higher sensitivity and selectivity. However, because DNA is released from both dead and living cells, PCR-based techniques may struggle to differentiate between non-viable and viable cells.

In the last fifteen years, CDs have been intensively investigated to improve their existing beneficial qualities. CDs' fluorescent features have got attention since they have unique qualities such as photostability, extended PL decay durations, excitation-dependent emission, and high fluorescence quantum yield (QY). These properties have led to the usage of CDs in sensing and imaging applications. CDs are used to detect harmful microorganisms and pathogens because of their great sensitivity, fluorescence characteristics, water solubility, and biocompatibility. First, the microorganisms are labeled with the fluorescence groups. Then, the number of bacteria is determined by the fluorescence intensity and finally estimated to fulfill the detection aim.

## Carbon quantum dots-based optical detection of pathogenic bacteria

CDs have clear benefits as fluorescent labeling materials for achieving high-sensitivity. In the identification of disease-causing bacteria, the characteristics of CDs outperform those of fluorescent probes. The most popular approach is to utilize CDs to identify harmful microorganisms directly for detection. CDs offer a narrower emission spectrum and more excellent light stability than traditional fluorophores, and are more biocompatible, brighter, and more specific. To detect ultrasensitive *Staphylococcus aureus* (*S. aureus*), Yang et al. developed breakable organosilica nanocapsules (BONs) encapsulated with carbon dots (CDs) as fluorescent labels. Cohydrolysis of tetraethyl orthosilicate and bis[3-(triethoxysilyl)propyl]disulfide resulted in core–shell CDs@BONs, which contained hundreds of CDs. To directly identify *S. aureus*, immunofluorescent nanocapsules (anti-*S. aureus* antibody-conjugated CDs@BONs) were developed. CDs were released by BONs using a basic $NaBH_4$ reduction method prior to fluorescent detection. Compared to traditional immunoassays using CDs as fluorescent markers, the fluorescent signals were amplified by two orders of magnitude due to hundreds of CDs encapsulated in each nanocapsule. At *S. aureus* concentrations of 1–200 CFU $mL^{-1}$, a linear range was obtained. CDs@BONs is also planned to be extended to other techniques, allowing for the identification of ultralow target concentrations.[70]

Without altering their emission characteristics or aptamer/antibody specificity, CDs may easily attach to aptamers/antibodies to produce functional CDs. Under single wavelength stimulation, CDs can be linked with aptamers. Because of their affinity for biomolecules, excellent specificity, and stability, aptamers have been widely employed in identification and detection applications. For example, for the detection of *Acinetobacter baumannii* (Ab), a ratiometric aptasensor was developed by Bahari et al. based on fluorescence resonance energy transfer (FRET) between graphene oxide (GO) as an acceptor and nitrogen-doped carbon nanodots (NCND) and ortho-phenylenediamines carbon dot (o-CD) as donor species. The modification o-CD with aptamer and the GO combined with NCND were used to fabricate a ratiometric fluorescence biosensor. Using EDC and NHS as activation agents, amine riched-NCND was covalently bound to the carboxyl group on the GO's edge in the first phase. Following that, o-CD with carboxylic groups loaded with amine-terminated DNA (o-CD-ssDNA) was adsorbed onto graphene oxide via the $\pi$-$\pi$-stacking interaction after covalent bonding by NHS-EDC. GO significantly inhibited the fluorescence of NCND and o-CD. When Ab bacteria are present, o-CD-ssDNA that is preferentially attached to the target bacteria is released from the GO. The quenched fluorescence of o-CD is turned-on but the fluorescence intensity of NCND is very minimally changed (Figure 1). With the concentration of bacteria varying from $2.0 \times 10^3$ to $4.5 \times 10^7$ CFU/mL, the fluorescence intensity ratio (I550 nm/I440nm) varied from 2.0 to 10.0. The low LOD was obtained at $3.0 \times 10^2$ CFU/mL (S/N = 3). The proposed aptasensor's ability to select Ab in urine samples was also proven with good results.[71] In another work, for sensitive and selective detection of *Escherichia coli* O157: H7 (*E. coli*), CDs were synthesized using a green one-step method. A microwave-assisted technique was used to prepare CDs from the orange peel as a carbon source. Under the excitation wavelength of 420 nm, the CDs emitted a bright green fluorescence. CDs tagged with aptamer (aptamer-CDs) and magnetic nanoparticles tagged with complementary DNA (cDNA-MNPs) were used to prepare a fluorescent probe (CDs-MNPs) for *E. coli*. With the addition of *E. coli*, the fluorescent intensity of the CDs-MNPs was decreased. The relationship between fluorescence intensity and *E. coli* concentration is linear. A detection range of 500–$10^6$ CFU/mL and a detection limit of 487 CFU/mL were obtained using this fluorescent probe. This approach was used to analyze *E. coli* in *E. coli*-infected milk samples, and the results were consistent with those obtained by plate-counting methods.[72]

A wireless electrochemical and luminescent sensor was constructed for bacteria detection using surface-coatable electrochemically formed fluorescent CDs synthesized from

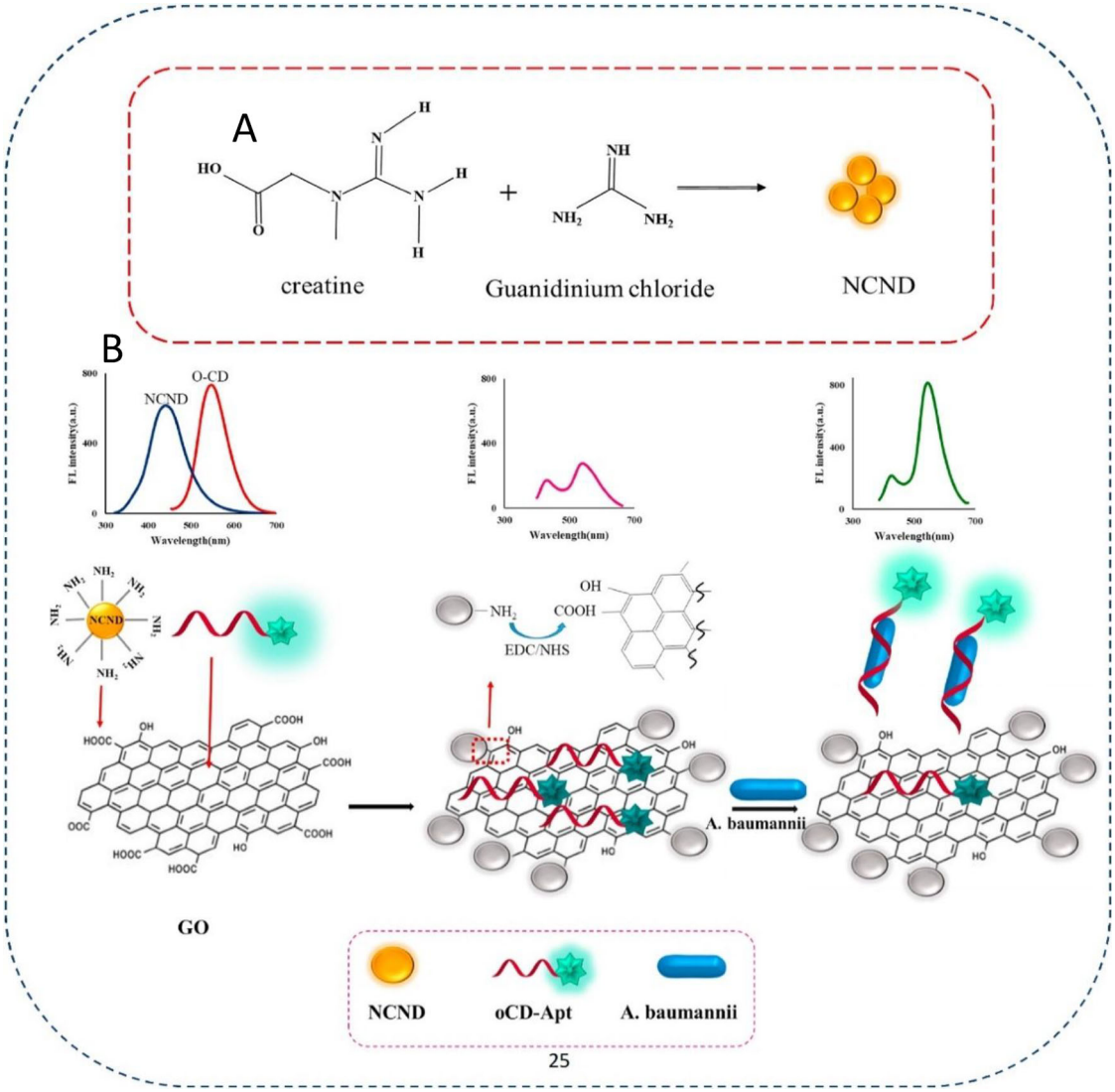



**Figure 1.** (A) Synthesis of nitrogen-doped carbon nanodots (NCND), (B) Assembly of the nanosystem and detection of *Acinetobacter baumannii*. Reprinted from Ref. [71] with permission from Elsevier.

a cationic polymer. By using catechol moieties on the fluorescent carbon dot (FCD) surface for photothermal-based antibacterial activity, the FCDs were further combined with NIR-responsive cesium tungsten oxide ($CsWO_3$). Because of the interaction between the cationic FCD surface and the anionic bacterial cell wall, the $CsWO_3$–FCD nanohybrids displayed significant fluorescence, which was quenched. The change in resistance value before and after interaction with bacteria might potentially be used to detect this interaction electrochemically. The LOD measured using the luminescent approach is 70 CFU/mL for *E. coli* and 131 CFU/mL for *S. aureus*. The electrochemical method was 10 CFU/mL for both bacterial strains, suggesting the electrochemical approach is more sensitive. Moreover, due to the photothermal conversion of $CsWO_3$, this nanohybrid produced photothermolysis of bacteria following NIR exposure, killing over 100% of *E. coli* and *S. aureus* with just 1 mg/mL of $CsWO_3$–FCD. As a result, this nanohybrid provides a unique technique for dual luminescent and electrochemical-based bacteria detection, as well as strong antibacterial activity.[73] The interactions of amphiphilic CDs with real bacterial cell membranes (rather than models) were also investigated, and the emission spectra of CDs were shown to be dependent on bacterial strain identification in terms of spectrum shift and peak intensity. This might lead to the identification of various bacteria species utilizing fluorescence spectroscopy and microscopy.[74] A different group used the unique interaction of CDs with bacterial membranes to identify bacteria in a biological context.[75]

In the fight against infectious illnesses and the spread of antibiotic-resistant bacteria, new technologies for detecting and killing harmful microorganisms are critical. The combination of silver nanoparticles (AgNP) and fluorescent carbon dots (FCDs) helps to detect synergic activity and determine antibacterial activity. However, the FCDs' fluorescence is quenched due to contact with AgNP, which is a significant restriction. To prevent the fluorescence quenching of the FCD-AgNP combination owing to Forster Resonance Energy Transfer (FRET), poly(vinylpyrrolidone) (PVP), and catechol chemistry (PVP@Ag:FCD) was employed. The enhancement of electrostatic interaction with the negatively charged bacterial surface and production of fluorescence

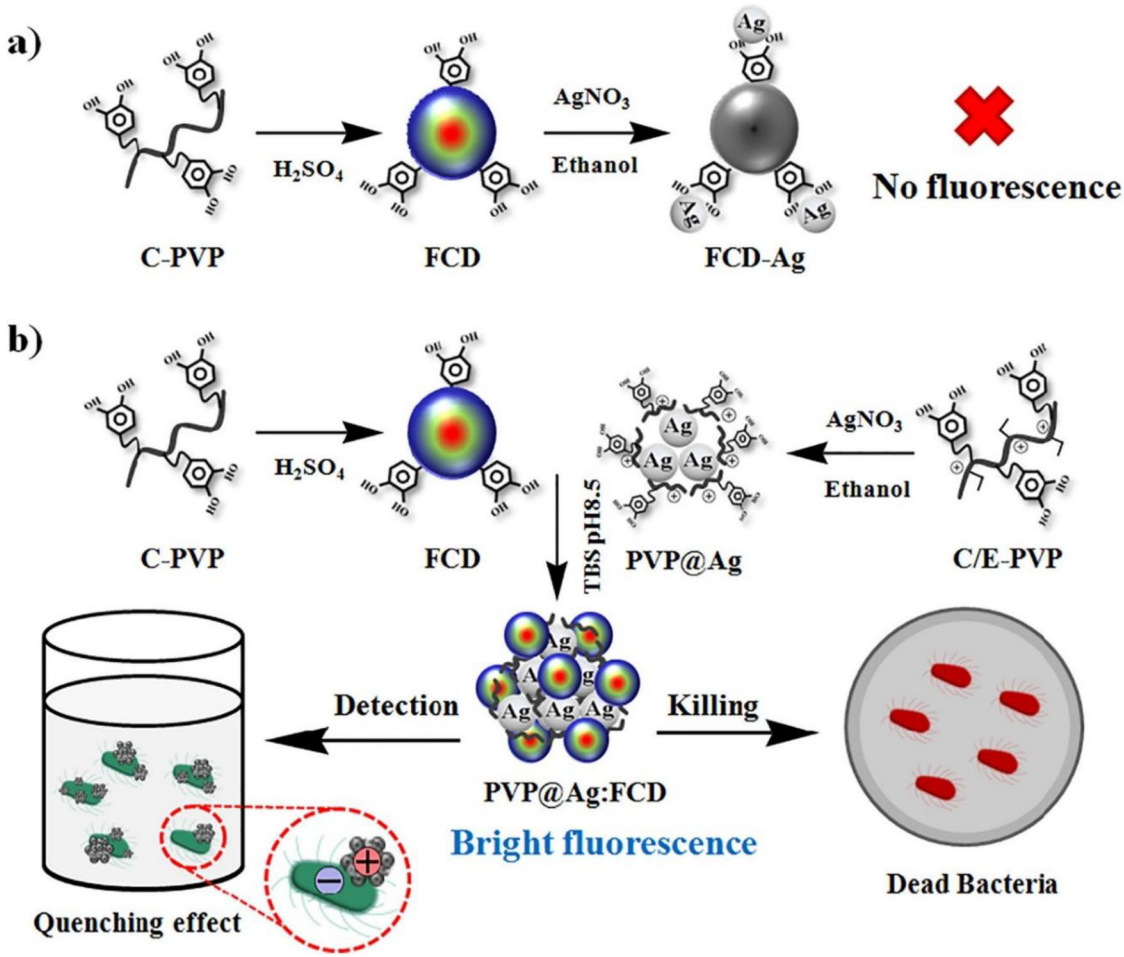



**Figure 2.** The illustration of poly(vinylpyrrolidone) modified silver nanoparticles capped carbon dots (PVP@Ag:FCD) synthesis steps and its function in detecting and killing efficiency against *S. aureus* and *E. coli*. Reprinted from Ref. [76] with permission from Elsevier.

quenching owing to accumulation quenching resulted in strong fluorescence that may be exploited for bacterial identification in PVP@Ag:FCD (Figure 2). In addition, the inclusion of silver nanoparticles in PVP@Ag:FCD resulted in a high bacterial killing efficiency against *S. aureus* and *E. coli* even at low quantities (0.1 mg/mL). The PVP@Ag:FCD system demonstrated easy, highly sensitive, and efficient identification and eradication of bacteria in polluted river water. As a result, this technology offers a promising way of detecting and eliminating germs in the future.[76]

Some of the optical biosensors based on CDs used for the detection of bacterial pathogens are given in Table 1. From Table 1, it is obviously seen that the most preferred synthesis method of CDs is solvothermal synthesis, using citric acid and ethylenediamine as precursors. Furthermore, most used approaches rely on the FRET "off-on" mechanism for optical detection of bacterial pathogens. The majority of the real samples used to evaluate the method's feasibility are water, juice, and milk samples.

## *Carbon quantum dots-based electrochemical detection of pathogenic bacteria*

Electrochemical biosensors have attracted a lot of attention because of their ability in providing quick, precise, and sensitive responses. Through different techniques, nanomaterials and nanocomposites in biosensors improve the sensitivity of detecting mechanisms and provide improved detection limits. Pangajam et al. developed an ultrasensitive electrochemical biosensor based on CD/ZnO/PANI conductive layers prepared by in situ polymerizations of aniline in the presence of CD/ZnO, which were utilized as modified electrodes for electrochemical detection of *E. coli* O157:H7. The sensitivity of the suggested sensor was twice that of the DNA-based biosensor described in the literature due to the increased conductivity of the nanocomposites. Furthermore, the exceptional electrical conductivity of CD/ZnO/PANI was discovered to improve the sensitivity of the detection of *E. coli*. The proposed electrochemical biosensor showed high selectivity and was effectively utilized to detect *E. coli* O157:H7 with a LOD of $1.3 \times 10^{-18}$ M in water samples.[83] Bacterial multi-resistance is driven by the extensive abuse of antibiotics, which implies that bacterial response to antibiotics in the treatment of infections must be developed in a rapidly detected and characterized manner. In one study, the bacterial response to antibiotic detection was achieved by creating an electrochemical biosensor based on graphene quantum dots (GQD) and an anoporous alumina membrane. Anti-*Salmonella* was combined with amino-modified glutaraldehyde GQD and immobilized to capture salmonella

**Table 1.** Application of CDs for optical detection of pathogenic bacteria.

| Carbon dot-based fluorecent material | Precursors | Synthesis method | Target bacteria | Recognition component | Mechanism of detection | Linear range CFU/mL | LOD CFU/mL | Application | References |
|---|---|---|---|---|---|---|---|---|---|
| B-PgP-functionalized FCDs | B-PgP | Solvothermal method | *S. aureus and E. coli* | Boronate ester bonding | FRET "off-on" | $10^1$–$10^7$ | – | contaminated river water | [77] |
| PVP@Ag:FCD | Catechol-quaternized PVP (C-PVP) | Solvothermal method | *E. coli* and *S. aureus* | PVP@Ag:FCD | FRET | $10^1$–$10^7$ | – | contaminated water | [76] |
| CDs@BONs | Citric acid and ethylenediamine | Solvothermal method | *S. aureus* | – | Breakable organosilica nanocapsule signal amplification | 1–200 | 1 | milk and orange juice | [70] |
| CDs | Citric acid and ethylenediamine | Solvothermal method | *P. aeruginosa* | Aptamer | FRET "off-on" | $10^1$–$10^7$ | 9 | Real water samples | [78] |
| Multicolor CDs | o-, m-, and p-phenylenediamines isomers | Solvothermal method | *S. aureus* | $Fe_3O_4$ + aptamer | Light-emitting mechanism | 50–$10^7$ | 8 | milk and juice | [79] |
| $NH_2$-CDs | Citric acid and polyethyleneimine | Solvothermal method | *E. coli* O157: H7 | Aptamer | FRET | $10^2$–$10^7$ cells/mL | 89 cells/mL | Milk | [80] |
| CDs | Polyethyleniminewith citric acid | Microwave pyrolysis | Klebsiella pneumoniae | DNA | Photoluminescence | – | – | Bacterial DNA | [81] |
| CDs@colistin | Di ammonium hydrogen citrate | Pyrolysis | *E. coli* | – | Photoluminescence | $3.81 \times 10^2$–$2.44 \times 10^4$ | 460 | Human urine, apple juice, and tap water | [82] |

Note: B-PgP-functionalized FCDs: Pluronic-grafted poly(dimethylaminoethyl methacrylate)–quaternized boronic acid functionalized fluorescent carbon dots.
Abbreviations: FRET: Förster resonance energy transfer; *P. aeruginosa*: *Pseudomonas aeruginosa*.

bacteria on a silanized nanoporous alumina membrane. The collection of bacteria on nanoporous membranes and the bacterial response to antibiotics could be monitored using impedance signals across nanoporous membranes. This nanoporous membrane and GQD-based electrochemical biosensor detected bacterial response to antibiotics in less than 30 minutes, with a detection limit as low as pM. It was possible to investigate the reaction of bacteria to antibiotics in a much faster and more convenient manner with previous techniques. The ability in the field of illness monitoring, the deletion of complete threats to food safety, and even hostile life have potential uses for investigating the dynamic effects of antibiotics on bacteria.[84] In another work, Savas et al. initiated an electrochemical sensing technique for the first time in the milk and human serum for extremely sensitive and specific determining of *Yersinia enterocolitica* (*Y. enterocolitica*). To this purpose, the activated GQDs were coated on a gold electrode before immobilization, and the bovine serum albumin (BSA) and ethanolamine (EA) unreacted carboxy groups were subsequently inactivated on the surface (Figure 3). The bacteria has been quantified by reducing $H_2O_2$ by GQDs. The bare gold electrode surface could not reduce $H_2O_2$ itself, whereas the GQD-modified gold electrode significantly reduced $H_2O_2$ and progressively generated signals of mA depending on the increasing concentration of GQD utilized. The GQD-modified electrode's improved electrocatalytic activity might be due to the close electrical contacts between Au and GQDs, enhancing electron transport. Bacterial detection was based on the degree of inhibited electron transfer of the laminated GQDs on the Au surface, which was hampered by developing an antigen-antibody combination on the GQD-modified Au electrode. As a result, when the concentration of Y. enterecolitica in the sample solution increased, the immunosensor's signal response decreased. The optimal assay conditions were first established, and the constructed immunosensor was then employed to detect the bacteria in milk and human serum. With high sensitivity ($LOD_{milk}$=5 CFU $mL^{-1}$ and $LOD_{serum}$= 30 CFU $mL^{-1}$) and specificity, the GQD-immunosensor quantified the *Y. enterocolitica* in a wide concentration range. Without pretreatment, the proposed technique may be utilized to identify any harmful bacteria in clinical and dietary samples.[85]

To detect Mycobacterium tuberculosis antigen (culture filtrate protein, CFP-10), a nanotriplex consisting of a GQD-coated $Fe_3O_4$@Ag ($Fe_3O_4$@Ag/GQD) core-shell nanostructure as the GCE modifier and AbD-AuNPs as the signal amplification label was used (Figure 4). Due to the roles played by the three nanomaterials the sensing platform employing the nanotriplex material has shown notable synergetic electrochemical performance: $Fe_3O_4$ has boosted the surface-to-volume ratio, GQDs have allowed more significant CAb loading on the electrode, and Ag improved its electric conductivity. The immunocomplexes were electrochemically activated in 0.1 M HCl at an optimized pre-oxidation potential of 1.3 V for 40 s to oxidize Au to $Au^{3+}$, which was then reduced to $Au^0$ during the DPV measurement. The immunosensor has been employed successively to

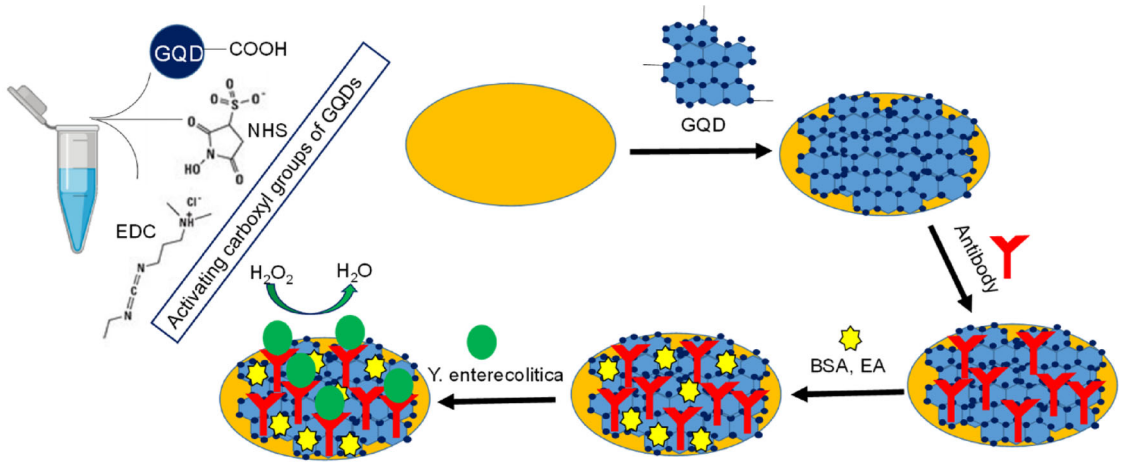



**Figure 3.** Illustration of graphene quantum dots (GQDs)-based immunosensor preparation for *Y. enterocolitica* detection. Reprinted from Ref. [85]

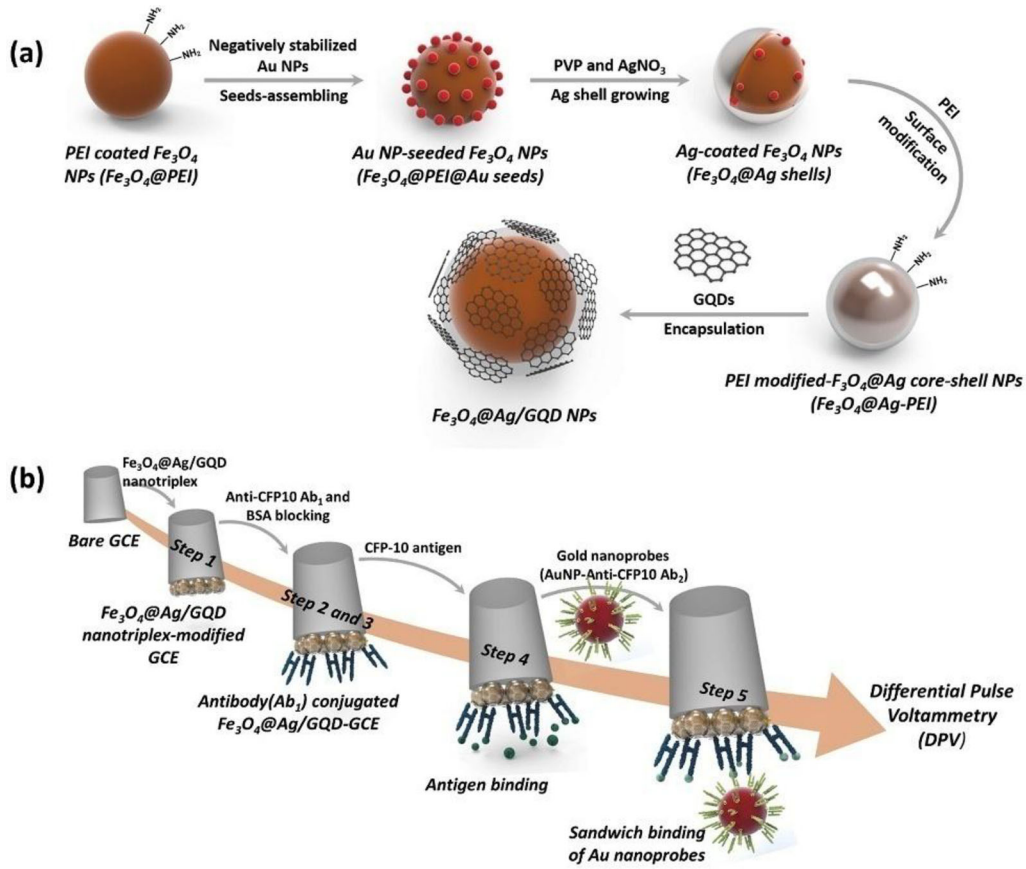



**Figure 4.** Schematic representative of the (a) graphene quantum dots-modified iron oxide/silver core-shell nanoparticles ($Fe_3O_4$@Ag/GQD NP) preparation; (b) the $Fe_3O_4$@Ag/GQD electrochemical *Mycobacterium tuberculosis* (Mtb) biosensor fabrication. Reprinted from Ref. [86] with permission from Elsevier.

test spiced human urine samples, showing broad linear distance (0.005–500 mg $mL^{-1}$ and a LOD of 0.33 ng $mL^{-1}$, respectively).[86]

A newly developed electrochemical DNA sensor based on screen-printed gold electrodes (Au-SPE) modified with CDs via drop-casting was designed to detect bacterial gene mutations. It detected a mutation in the cystic fibrosis transmembrane regulator (CFTR) gene, which can be traced back to the pathogen Helicobacter pylori. A redox indicator was used to detect a single nucleotide polymorphism (F508del mutation in the CFTR gene) in human DNA extracted from blood cells. Authors found no evidence of the F508del gene mutation but did find traces of a synthetic sequence complementary to the gene.[87]

Although the electrochemical technique is sensitive and simple for bacterial detection, there are no more papers for electrochemical detection of pathogenic bacteria using CDs. Other nanoparticles such as Au NPs, metal oxide NPs and so on were used that are not related to our subject.

## Carbon quantum dots-based virus detection

Carbon-based dots (CDs), known as zero-dimensional (0 D) nanomaterials, have been attracting increasing attention in sensing and bioimaging due to their unique electronic and optical properties.[88] CDs-based lateral flow assays, solution-based assays in optical detection, microfluidics assays, and various electrochemical techniques such as cyclic voltammetry, differential pulse voltammetry, and electrochemical impedance spectroscopy applied electrochemical sensors have all become useful tools in the field of virus detection (see Figure 5).

### *Carbon quantum dots-based optical detection of viruses*

The appropriate, fast, and sensitive identification of viruses can be achieved by combining CDs with technical instruments. As an antigen-antibody binding example for virus detection using microfluidics as a platform and CDs as fluorescent labels, Chunduri et al. developed a microwell-plate and microchip-based sandwich assay to detect p24 antigen, which is the most abundant viral protein of human immunodeficiency virus (HIV-1).[89] Streptavidin was attached to CDs (CD-SA) after carbon dots were synthesized using a single-step hydrothermal process. The schematic representation for the detection of HIV-1 p24 antigen CD-SA conjugates is shown in Figure 6. The concept of the carbon dot-based immunoassay (CDIA) is to capture HIV-1 p24 using capture-antibody coated microtiter wells, which are subsequently sandwiched between secondary biotinylated anti-p24 antibodies. The biotinylated antigen-antibody complex is recognized and captured by the CD-SA conjugates. The fluorescence signal of the sandwiched complex was captured and quantified after thorough washing. For CDIA, a wide linear detection range of 20–1000 pg/mL was found. CDIA also found no false-positive results in identifying HIV-1 p24 antigen in HIV-negative plasma samples. Notably, the authors extended this bench-top CDIA to develop a microfluidic carbon dot immunoassay ($\mu$CDIA) to provide better health care through point-of-care (POC) tests. $\mu$CDIA exhibited analytical sensitivity in the range of 30–1000 pg/mL with the detection limit of 30 pg/mL. The researchers also determined that the cost of $\mu$CDIA is 2.71$, which is less expensive than the cost of a traditional ELISA, which is 9.50$. The projected overall cost of $\mu$CDIA detection is 3–4 times lower than detection utilizing a traditional ELISA platform. Furthermore, both CDIA and $\mu$CDIA can be easily converted to a lab-on-a-chip architecture for usage in resource-constrained environments.

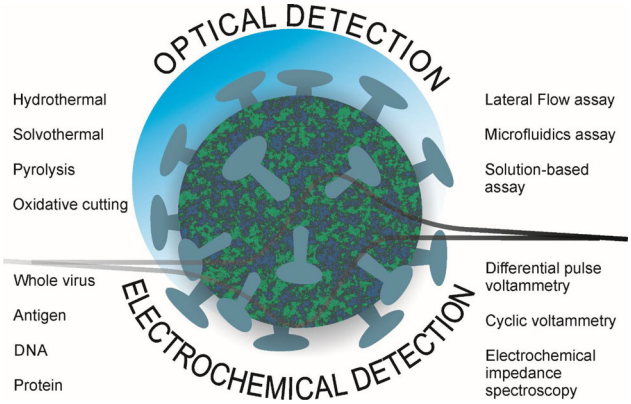



**Figure 5.** The used methods for synthesis of carbon dots (CDs), and the type and method of assay for optical detection and electrochemical detection of whole virus, antigen, DNA, and protein of viruses.

Various carbon-based materials (such as graphene-related materials) are being intensively evaluated for biosensing applications based on energy transfer due to their nearly universal, long-range, and extremely effective photoluminescence quenching characteristics.[90–92] In this regard, carbon nanoparticle oxide (CNPs) has been employed as an energy acceptor by taking advantage of their large light-absorption window, as described earlier by Ye et al..[93] Using dual-probe fluorescent silver nanoclusters (AgNCs), the authors developed a label-free biosensor for detecting HIV-DNA sequences. CNPs oxide was made from candle soot oxidation and utilized as a fluorescence quencher probe. The fluorescence resonance energy transfer (FRET) based on AgNCs and carbon nanoparticle oxide was used for sensing process. The strategy of dual AgNCs probes was applied to

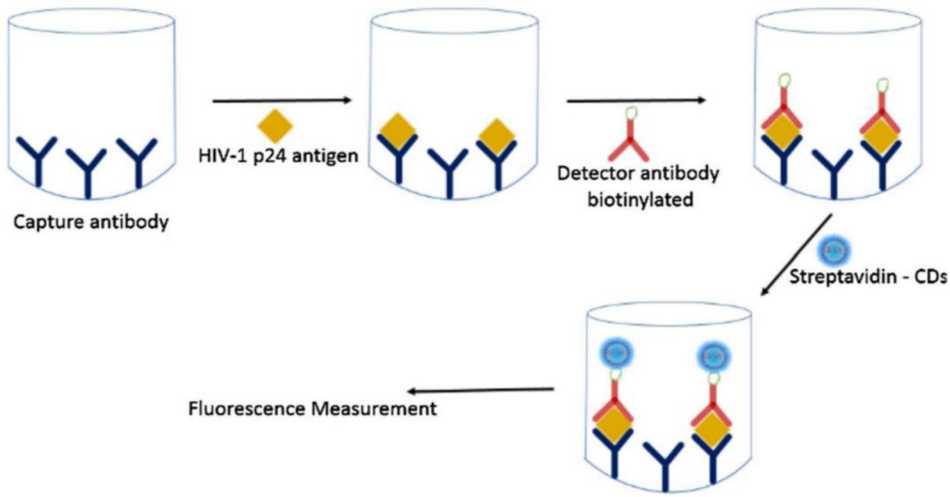



**Figure 6.** Schematic illustration for the detection of human immunodeficiency virus antigen (HIV-1 p24) by streptavidin coated carbon dots (CD-SA) conjugates. Reprinted from Ref. [89] with permission from Springer Nature.

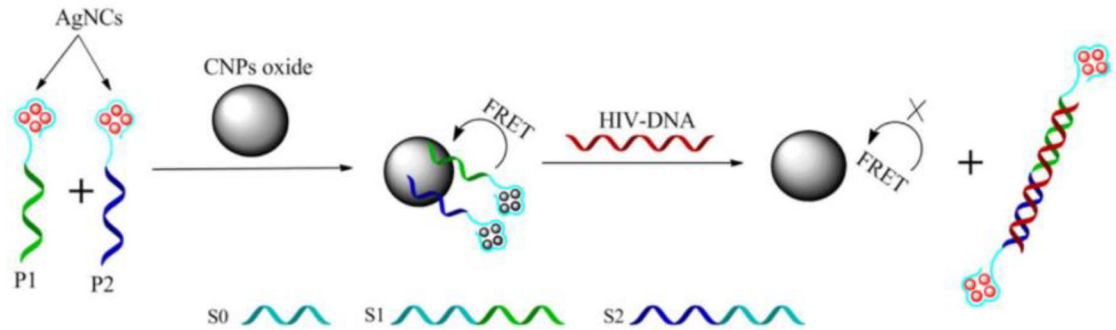



**Figure 7.** The schematic sensing module for the sensing of human immunodeficiency virus DNA (HIV-DNA) based on fluorescence resonance energy transfer (FRET) from DNA-protected silver nanoclusters (AgNCs) to carbon nanoparticle (CNPs) oxide. Reprinted from Ref. [93] with permission from Elsevier.

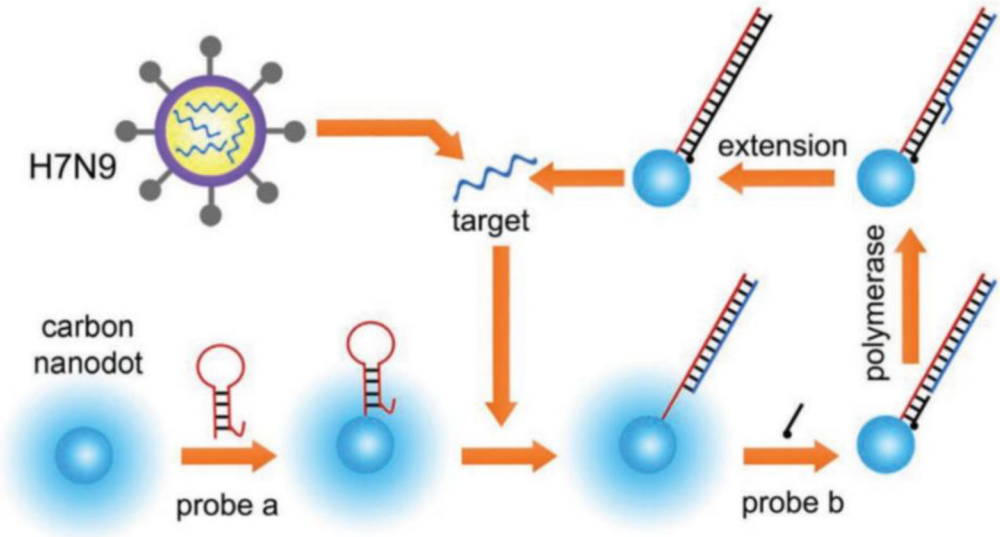



**Figure 8.** Illustration of the carbon nanodot–based amplified detection of virus DNA. Reprinted from Ref. [94] with permission from Wiley-VCH.

improve the detection sensitivity by using dual-probe capturing the same target DNA in a sandwich mode and as the fluorescence donor, and using CNPs oxide as the acceptor. In the presence of target DNA, dsDNA hybridizes, causing the ssDNA-AgNCs probes to desorb from CNPs oxide and the AgNCs to recover fluorescence in an HIV-DNA concentration-dependent manner. In an aqueous buffer, HIV-DNA can be identified in the range of 1–50 nM with a detection limit of 0.40 nM, according to the results. Figure 7 shows a schematic sensing module for HIV-DNA sensing based on FRET from DNA-protected AgNCs to CNPs oxide.

Nucleic acid amplification tests (NAAT) for the diagnosis and management of viral infections are widespread because they enable virus load determination. In recent research, a CDs-based fluorescent method was presented for an isothermal strand displacement amplification viral DNA analysis using deoxyribonucleic acid polymerase I purified from Bacillus stearothermophilus (*Bst* DNA polymerase), allowing for a double-strand displacement of DNA (dsDNA) at room temperature.[94] The designed DNA hairpin structured "probe a" was immobilized on the surface of CDs via the 3′ amino group in the performed study after the hydrothermal synthesis, successful purification, and characterization processes of CDs. “Probe a” has two recognition regions for target DNA, but “probe b” has its recognition sequence hidden in the hairpin’s stem. The “probe b” recognition sequence, on the other hand, may be exposed to hybridization events after interaction with the target sequence. The fluorescence emission of CDs is quenched because "probe b" is tagged with black hole quencher (BHQ). In *Bst* DNA polymerase, on the other hand, extension and strand displacement reactions occur, releasing target DNA. With extra "probe a" on the surface of CDs, the recycled sequence may be hybridized, and further CDs can be quenched. Figure 8 shows an illustration of viral DNA amplified detection using carbon nanodots.

Furthermore, cyclic strand displacement polymerization and highly luminescent CDs significantly increased sensitivity. The concentration of target DNA may be determined using this method by evaluating the significantly decreased fluorescence. The limit of detection values for the hemagglutinin7 (H7) and neuraminidase 9 (N9) genes, respectively, were estimated as $4.6 \times 10^{-15}$ and $3.4 \times 10^{-15}$ M. Furthermore, the suggested technique is extraordinarily selective and capable of recognizing target sequences in biological samples, indicating that it has a wide range of potential applications in biomedical research and clinical diagnostics.

In recent years, the development of magnet-nanoparticles (MNPs), which offers wide ranging opportunities in drug

and gene delivery, magnetic separation, diagnostics, and in vivo imaging, has had revolutionary impacts on pharmaceuticals analysis, food safety, environmental monitoring, clinical diagnostics, health monitoring, fundamental biological research, and biomedical applications.[95] Zarei-Ghobadi et al. suggested an HTLV I-detection genosensor based upon photoluminescence in the presence of iron magnetic nanoparticle capped Au($Fe_3O_4$@Au), with a photoluminescent quantity of fluorescent carbon dots.[96] They created two specialized probes for a unique long T-lymphotropic genome type 1 sequencing region. One of the probes was linked to the CDs covalently. In the absence of the target, two mechanisms were used to adsorb the CDs-probe on $Fe_3O_4$@Au's surface, which resulted in the fluorescent emission of the CDs being quenched. The fluorescence emission of CDs was recovered in the presence of a target since double-stranded DNA cannot adsorb on the $Fe_3O_4$@Au. Also, $Fe_3O_4$@Au can adsorb the unhybridized oligonucleotides and improves the accuracy of detection. Figure 9 provides a schematic representation of the DNA target detection procedures. The Basic Local Alignment Search Tool (BLAST) search confirmed the specificity of the proposed biosensor and examined it by exposing it to other virus targets. The biosensor's experimental detection limit was lower than 10 nM with a linear range from 10 to 320 nM.

Since the quantity of target species in a complex matrix is very low for accurate sensing, researchers explore more effective and sensitive multifunctional materials for separating and detecting viruses in clinical diagnosis. As an interesting study for the use of MNPs in virus detection, a magnetic/plasmonic-assisted fluoro-immunoassay system for the detection of influenza virus using magnetic-derivatized plasmonic molybdenum trioxide quantum dots (MP-$MoO_3$QDs) as the plasmonic/magnetic agent and fluorescent graphitic carbon nitride quantum dots (gCNQDs) as the monitoring..[97] The MP-$MoO_3$QD and the gCNQD surface were conjugated correspondingly with specific influenza A virus antibody (see Figure 10). In the presence of influenza A virus (as the test virus), a core-satellite immunocomplex is formed between the antibody-conjugated nanomaterials (Ab-MP-$MoO_3$QDs and Ab-gCNQDs) and their interaction resulted in the modulation and gradual enhancement of the fluorescence intensity of the detection probe with the influenza virus concentration-dependent increase. Furthermore, no photoluminescence (PL) change was observed in the absence of the influenza A virus. The detection limits for Influenza virus A/New Caledonia (20/99/IVR/116) (H1N1) in deionized water and human serum were 0.25 and 0.9 pg/mL, respectively. The limit of detection for clinically isolated influenza virus A/Yokohama (110/2009) (H3N2) was determined to be 45 PFU/mL in the range of 45–25,000 PFU/mL.

In another research, a different fluoroimmunoassay has been reported for influenza virus using sulfur-doped graphitic carbon nitride quantum dots (S-gCNQDs) coupled with $Ag_2S$ nanocrystals.[98] The researchers have synthesized S-gCNQDs by a single-source precursor in a one-step solvothermal process. The prepared S-gCNQDs and $Ag_2S$ nanocrystals were applied as innovative functional materials to fabricate a biosensor for virus detection based on the conjugation of specific anti-human influenza A monoclonal antibodies the S-gCNQDs and $Ag_2S$ NCs, respectively. The rationale behind the sensing mechanism is the interaction of the S-gCNQDs/$Ag_2S$-labeled antibody resulted in forming a nanosandwich structure, which is accompanied by the

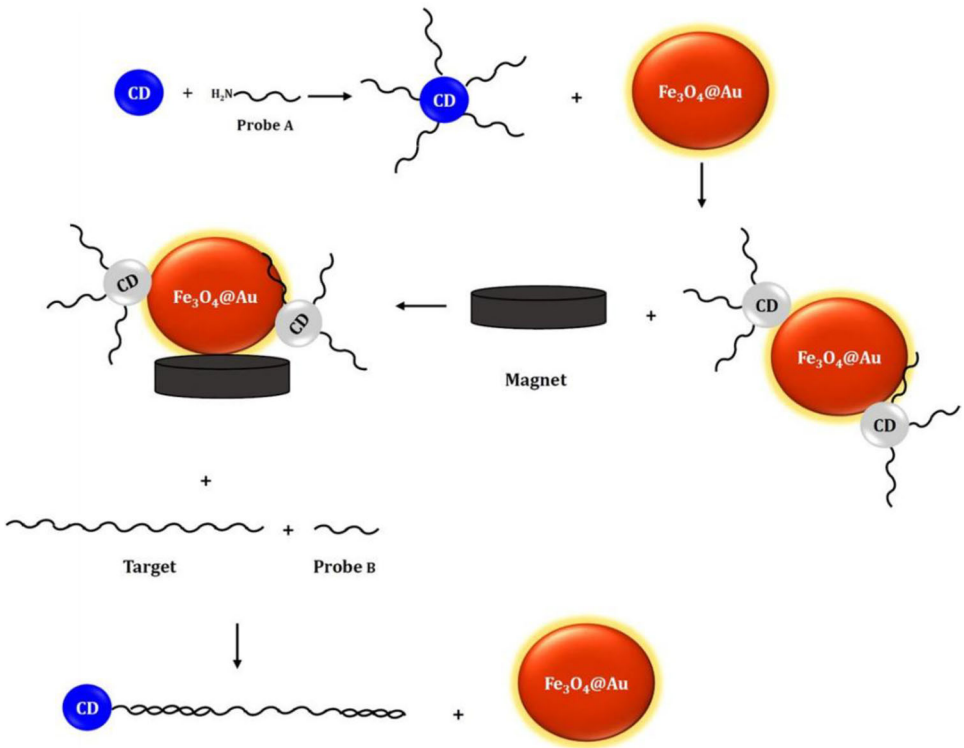



Figure 9. Schematic illustration of the detection steps of DNA target. Reprinted from Ref. [96] with permission from Nature Publishing Group.

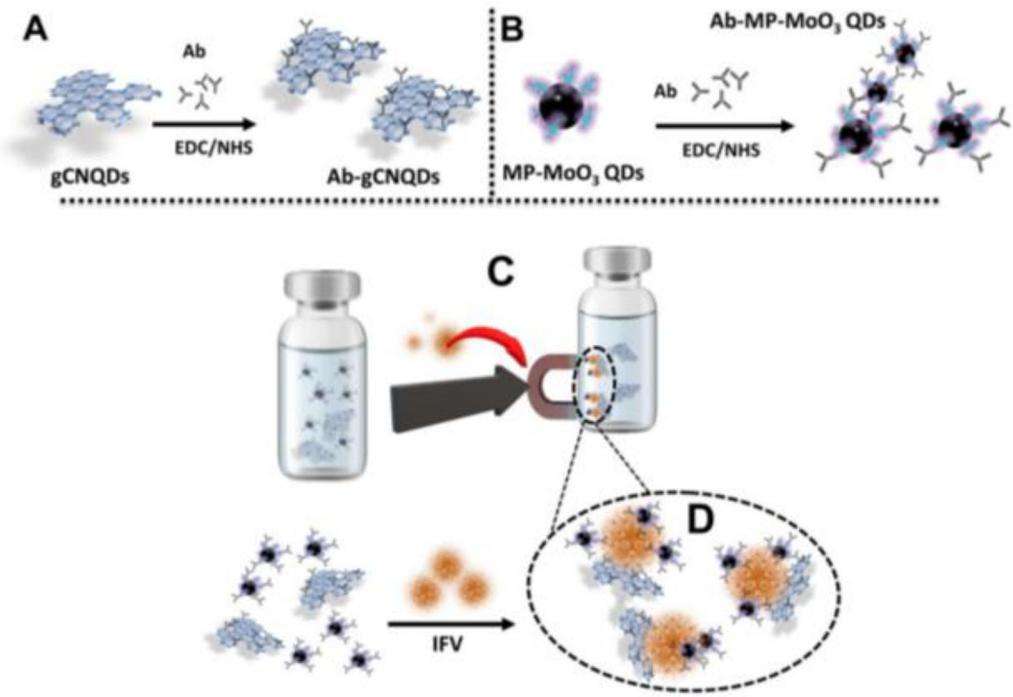



**Figure 10.** (A) Antibody conjugation to fluorescent graphitic carbon nitride quantum dots (gCNQDs) via EDC/NHS chemistry. (B) Antibody conjugation to MP-$MoO_3$ QDs via EDC/NHS chemistry. (C) Magnetic separation and purification step upon target virus addition (D) Core-satellite immunocomplex of gCNQDs and magnetic-derivatized plasmonic molybdenum trioxide quantum dots (MP-$MoO_3$QDs) in the presence of influenza virus. Reprinted from Ref. [97] with permission from Elsevier.

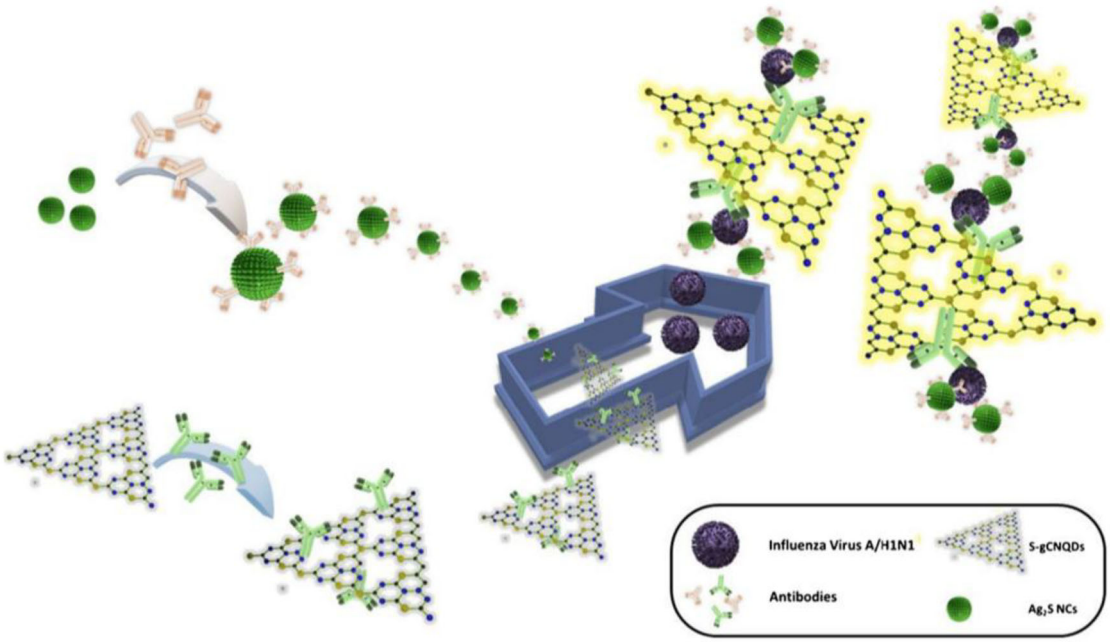



**Figure 11.** Schematic representation of the sulfur-doped graphitic carbon nitride quantum dots (S-gCNQDs) and silver disulfide nanocrystals ($Ag_2S$) modification with antibody and the detection protocol by sandwich nanostructure formation in the presence of target influenza A virus. Reprinted from Ref. [98] with permission from Springer Nature.

fluorescence enhancement of the S-gCNQDs in the presence of the influenza A virus. A schematic representation of the sensing principle is shown in Figure 11. The fluorescence intensity shows a linear change with the concentration of the influenza A virus (H1N1) between 10 fg/mL and 1.0 ng/mL with a LOD of 5.5 fg/mL. The assay was applied to the clinically isolated influenza A virus (H3N2/Yokohama) mixed with human serum. The obtained LOD was 100 PFU/mL within the detection range of $10^2$–$5 \times 10^4$ PFU/mL for the H3N2 virus.

Xu et al. demonstrated an effective method to assemble silanized carbon dots (CDs) with porous silica templates to produce fluorescent CD-based silica (FCS) colloids with uniformly covered CDs throughout the silica matrix.[99] As an absorbent host, dendritic silica spheres with highly accessible center-radial pores were used, establishing Si–O linkages

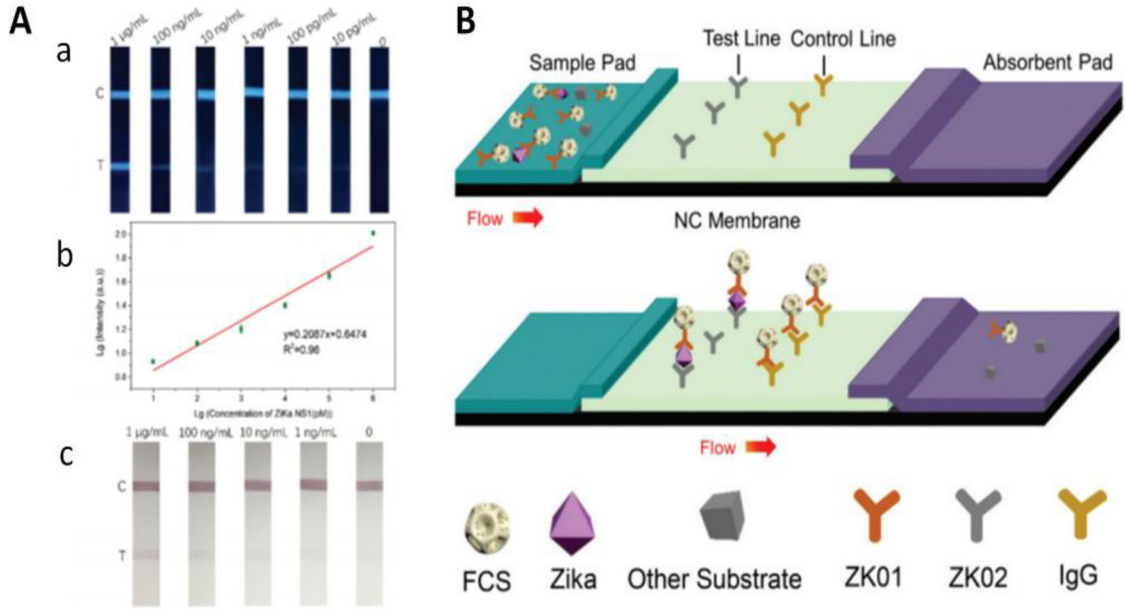



**Figure 12.** (A) The virus detection results by the proposed assay and comparison with AuNPs-based assay. (a) Fluorescence pictures of the lateral flow test strips at different concentrations of Zika NS1 protein solution. (b) The linear response of the test strip for Zika NS1 protein detection (c) Photographs of the gold nanoparticles (AuNP)-based lateral flow test strips at different concentrations of Zika NS1 protein solution. (B) Schematic representation for the Zika NS1 protein detection using the immunofluorescent carbon dots (iFCS)-based lateral flow immunoassay. Reproduced from Ref. [99] with permission from Royal Society of Chemistry.

with silane to the silanized CDs. The convenient amount of CDs on the inner surface of dendritic silica spheres is beneficial for the maximum fluorescence intensity of FCS colloids. High-quality silanized CDs endow multiple CD embedded silica spheres with excellent properties, including good fluorescence performance, excellent colloidal/optical stabilities, and convenient biofunctionalization. Figure 12 shows a schematic depiction of the sensing concept. A very sensitive, specific, and robust test on the Zika NS1 protein with a visual limit of 10 pg $mL^{-1}$ has been performed for the detection of the Zika virus in clinical samples by the combination of these FCS colloids with the lateral flow strip platform. The authors also produced and evaluated the standard LFA based on Au NP for Zika NS1 protein. When compared to AuNPs-based LFA, the detection limit of the proposed LFA was shown to be 100 times lower.

High-affinity carbon nanomaterial-nucleobase interaction, which can be used for DNA-directed assembly and sensing, is still challenging. Li et al. used a one-step solvent, thermal approach to prepare boron and nitrogen co-doped single-layered graphene quantum dots (BN-SGQDs).[100] Density functional theory (DFT) simulation supported the usage of BN-SGQDs as a high-affinity platform for nucleic acid detection and imaging in living cells. The Yellow-emitted BN-SGQDs serve as an energy donor in the nucleic acid detection (FRET) method due to their superior absorption and photoluminescent capabilities. In addition to the dynamic invasions of human Immunodeficiency Virus (HIV) DNA, which is based on the BN-SGQDs sensing platform, HeLa cells have effectively been introduced. In complex biological samples, this platform showed potential for biosensing. Figure 13 shows a schematic depiction of the sensing concept.

As an advantage, carbon dots/$SiO_2$ nanosphere (CSNs) make the test strips more sensitive and they have a longer assay lifetime. Xu et al. have reported an immunofluorescent CSNs-based LFA for sensitive detection of severe fever with thrombocytopenia syndrome virus (SFTSV) (see Figure 14).[101] A visual LOD value of 10 pg/mL of SFTSV nucleoprotein was found in the suggested LFA. The sensitivity of this assay is two times higher than that of the colloidal gold-based lateral flow test strip. Besides, the developed lateral flow test assay was evaluated in real samples of human serum of patients with satisfactory results.

Table 2 shows the summary of different approaches for CDs synthesis and optical detection of viruses. It is seen that mostly preferred synthesis method is solvothermal synthesis, and the most used precursor is citric acid. It is also important to remark that mostly proteins, specific antigens, or DNA of viruses are the target analytes despite whole virus detection. Therefore, DNA probes to target DNA and specific antibodies to proteins are widely used as recognition units in designed sensing principles. In addition, it was observed that all these recently reported studies provided promising detection limits.

### *Carbon quantum dots-based electrochemical detection of viruses*

For accurate and quick diagnosis, electrochemical sensing-based systems must be adapted to identify viruses. Furthermore, integrating CDs into these detection techniques and incorporating them into composite nanomaterials has enhanced detection limits, allowing for the integration of point-of-care devices to the personalized medicine.[102] In this regard, Wang et al. developed an electrochemical

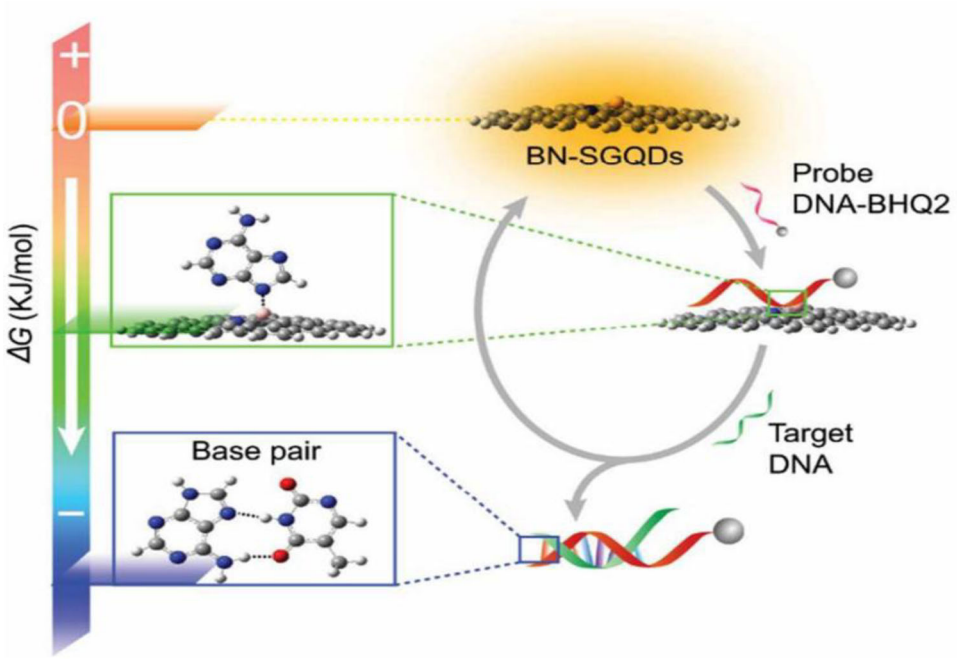



**Figure 13.** Schematic illustration of changes in Gibbs free energy of nucleobases and boron and nitrogen co-doped single-layered graphene quantum dots (BN-SGQDs) in the nucleic acid sensing process and the target DNA-induced fluorescence enhancing of BN-SGQDs + P complex. BHQ2: Black Hole Quencher 2 (which has a high absorption coefficient at 560 nm). P: probe DNA-BHQ2. Reprinted from Ref. [100] with permission from Royal Society of Chemistry.

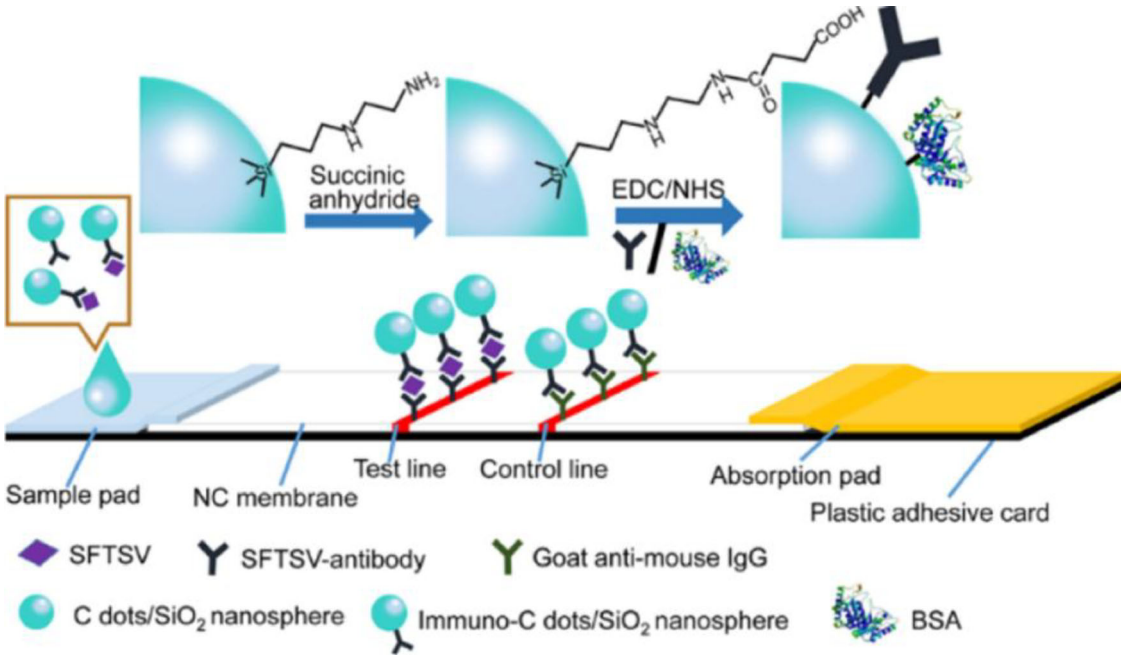



**Figure 14.** Schematic representation of functionalization process for carbon dots/$SiO_2$ nanosphere (CSNs) and lateral flow assay (LFA) principle. Reprinted from Ref. [101] with permission from ACS.

sandwich-based immunoassay for sensitive detection of avian leukosis virus subgroup J (ALVs-J) combining graphene quantum dots (GQDs) and Cu-apoferritin nanoparticles to amplify the signal.[103] After compounding with $Fe_3O_4$, GQDs were employed to conjugate primary ALVs-J antibodies (Ab1) and immobilize secondary ALVs-J antibodies (Ab2). As electroactive probes, Cu-apoferritin nanoparticles were immobilized onto $Fe_3O_4$@GQDs hybrids. After the well-known sandwich-type assembly, Cu was released from the apoferritin cavity and then detected by differential pulse voltammetry (DPV). Because GQDs have such a large surface area, they were able to load a large number of antibodies onto the immunosensor, substantially increasing the signal. Furthermore, the addition of Cu-apoferritin nanoparticles greatly enhanced the loading quantity of electroactive probes, amplifying the signal. Figure 15 depicts a schematic depiction of the sensing concept. To demonstrate the protocol's signal amplification feature, the performance of several labels was examined in detail. As a result, the developed immunosensor showed excellent analytical performance for

**Table 2.** Studies on carbon-based nanodots for optical detection of viruses.

| Carbon-based fluorecent material | Precursors | Synthesis Method | Quantum Yield | Target Virus | Target Analyte | Recognition Matrix | Linear Range | LOD | Application | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Carbon dots | Citric acid and ethylenediamine | Hydrothermal method | 95.7% | HIV | p24 antigen | Biotinylated anti-p24 antibody | 30–1000 pg/mL | 30 pg/mL | Human plasma samples | [89] |
| Carbon nanoparticles oxide | Candle soot | Oxidation | — | HIV | HIV-DNA sequences | ssDNA-AgNCs | 1–50 nM | 0.40 nM | PBS solution pH 7.5 | [93] |
| carbon dots | Citric acid and thiourea | Solvothermal method | 43.02% | H7N9 | Hemagglutinin7 (H7) gene and neuraminidase 9 (N9) | DNA | $10 \times 10^{-15}$–$10 \times 10^{-12}$ M | $4.6 \times 10^{-15}$ M $3.4 \times 10^{-15}$ M | Diluted serum samples | [94] |
| Carbon dots | o-phenylenediamine | Hydrothermal method | — | HTLV-I | DNA | CDs-DNA probe | 10–320 nM | <10 nM | — | [96] |
| Graphitic carbon nitride quantum dots | Citric acid and melamine | | 44% | H1N1 H3N2 | H1N1 H3N2 | Specific antibody against influenza A virus | 0–$10^5$ pg/mL for H1N1 45–25,000 PFU/mL for H3N2 | 0.25 and 0.9 pg/mL for H1N1 45 PFU/mL for H3N2 | Deionized water and human serum | [97] |
| Sulfur-doped graphitic carbon nitride quantum dots | Citric acid and 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole | Solvothermal method | ~ 33% | H1N1 H3N2 | H1N1 H3N2 | Specific antihuman influenza A monoclonal antibody conjugated S-gCNQDs | 10 fg/mL–1.0 ng/mL for H1N1 $10^2$–$5 \times 10^4$ PFU/mL for H3N2 | 5.5 fg/mL for H1N1 100 PFU/mL for H3N2 | Clinical samples and human serum | [98] |
| Carbon dots | Citric acid and N-$\beta$-(aminoethyl)-$\gamma$-aminopropyltrimethoxysilane | Solvothermal method | — | Zika virus | Zika NS1 protein | ZK01 antibody conjugated fluorescent CD-based silica (FCS) spheres | 0 pg/mL − 1 μg/mL | 10 pg $mL^{-1}$ | Clinical samples and human serum | [99] |
| Boron and nitrogen co-doped single-layered graphene quantum dots (BN-SGQDs) | p-Phenylenediamine and 3-Formylphenylboronic acid | Solvothermal method | 36.5% | HIV | HIV-DNA | BN-SGQDs-DNA probe | 0 − 20 nM | 0.5 nM | Tris-HCl buffer (pH: 7.4) and living HeLa cells | [100] |
| Carbon dots | Citric acid and N-$\beta$-(aminoethyl)-$\gamma$-aminopropyl trimethoxysilane | Solvothermal method | 56.3% | SFTSV | SFTSV nucleoprotein | carbon dots/$SiO_2$ nanosphere-Antibody Conjugates | 10 pg/mL − 1 μg/mL | 10 pg/mL | SFTSV standard solution and human serum | [101] |

Abbreviations: HIV: human immunodeficiency virus; H7N9: (hemagglutinin7 gene and neuraminidase 9 gene) Avian influenza A virus; HTLV-I: human T-lymphotropic virus type 1; H1N1: Influenza virus A/New Caledonia (20/99/IVR/116); H3N2: Clinically isolated influenza virus A/Yokohama (110/2009); DNA: deoxyribonucleic acid; SFTSV: thrombocytopenia syndrome virus.

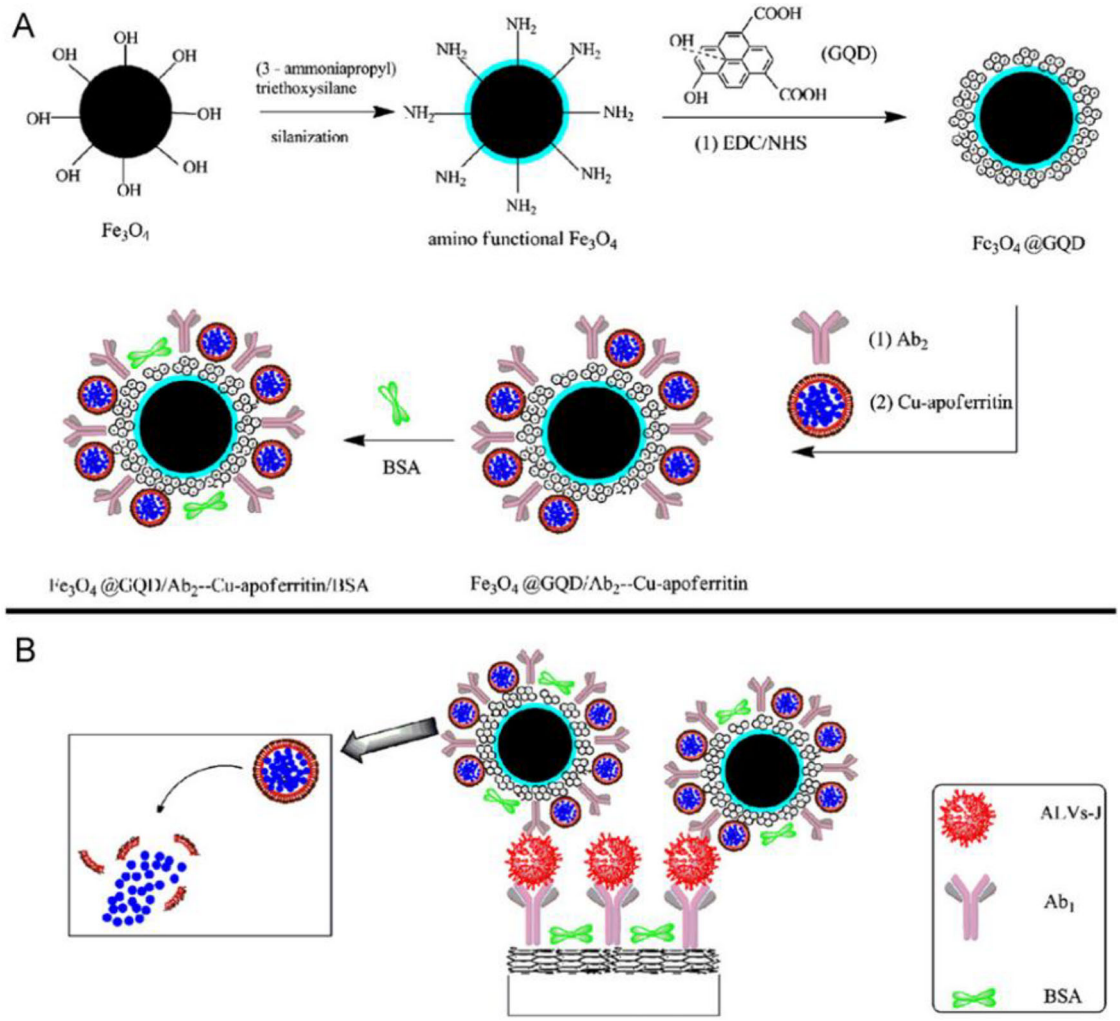



**Figure 15.** Schematic representation of the preparation of bovine serum albumin (BSA) blocked, iron oxide nanoparticles and graphene quantum dots combined antibody/Cu-apoferritin nanoparticles $Fe_3O_4$@GQDs/$Ab_2$–Cu-apoferritin/BSA (A) and immunosensor (B). Reprinted from Ref. [103] with permission from Elsevier.

the detection of ALVs-J in the range of $10^{2.08}$–$10^{4.50}$ $TCID_{50}$/mL ($TCID_{50}$: 50% tissue culture infective dose) with a limit of detection of 115 $TCID_{50}$/mL (S/N = 3), as well as high sensitivity, reproducibility, and stability.

In some circumstances, the use of nanocomposites for electrochemical virus detection also gives a particular enhanced surface area capable of loading a large number of antibodies to entrap the antigen. For ultrasensitive and selective detection of hepatitis C virus core antigen (HCV), Valipour and Roushani used silver nanoparticles (AgNPs) and thiol graphene quantum dots (GQD-SH) as nanomaterials.[104] The AgNPs/GQD-SH nanocomposite was used as a platform for covalently attaching antibodies to the working electrode's surface. A schematic representation of the sensing principle is shown in Figure 16. The decrease in the electrochemical signal is evaluated as the specific recognition between antibodies and antigens. It's also worth noting that riboflavin was the redox probe utilized to develop the suggested immunosensor. The suggested electrochemical immunosensor has a linear range of 0.05 pg $mL^{-1}$ to 60 ng $mL^{-1}$ and a LOD of 3 fg $mL^{-1}$. The developed immunosensor was also utilized to evaluate a blood sample, yielding accurate HCV core antigen detection results.

The electroactive polymer nanospheres (PS) made from infinite coordination polymers of ferrocenedicarboxylic acid can be used as a carrier and electronic mediator to load carbon-based composite materials. In this regard, utilizing a signal amplification method, a sensitive label-free electrochemical immunosensor for quantitative detection of hepatitis B surface antigen (HBsAg) was described.[105] Surfactant-free AuPdCu ternary nanoparticles (AuPdCu/N-GQDs) were produced by using nitrogen-doped graphene quantum dots (N-GQDs). The PS enhanced the number of loading antibodies due to high biocompatibility and the rapid electron transfer rate to the electrode interface ascribed to the ferrocene unit. Figure 17 shows a scheme representing the sensing concept. The electrical immunosensor developed exhibited good sensitivity and stability for HBsAg detection under optimum circumstances. A linear correlation was established between electrochemical signals, with a detection limit of 3.3 fg/mL, and the HBsAg concentration range between 10 fg/mL and 50 ng/mL. In addition, the proposed immunosensor was highly selective, reproductive, stable, and satisfactory in human serum samples.

GQDs may be helpful to nanomaterials as an aptamer platform through $\pi$–$\pi$ stacking interactions, which help

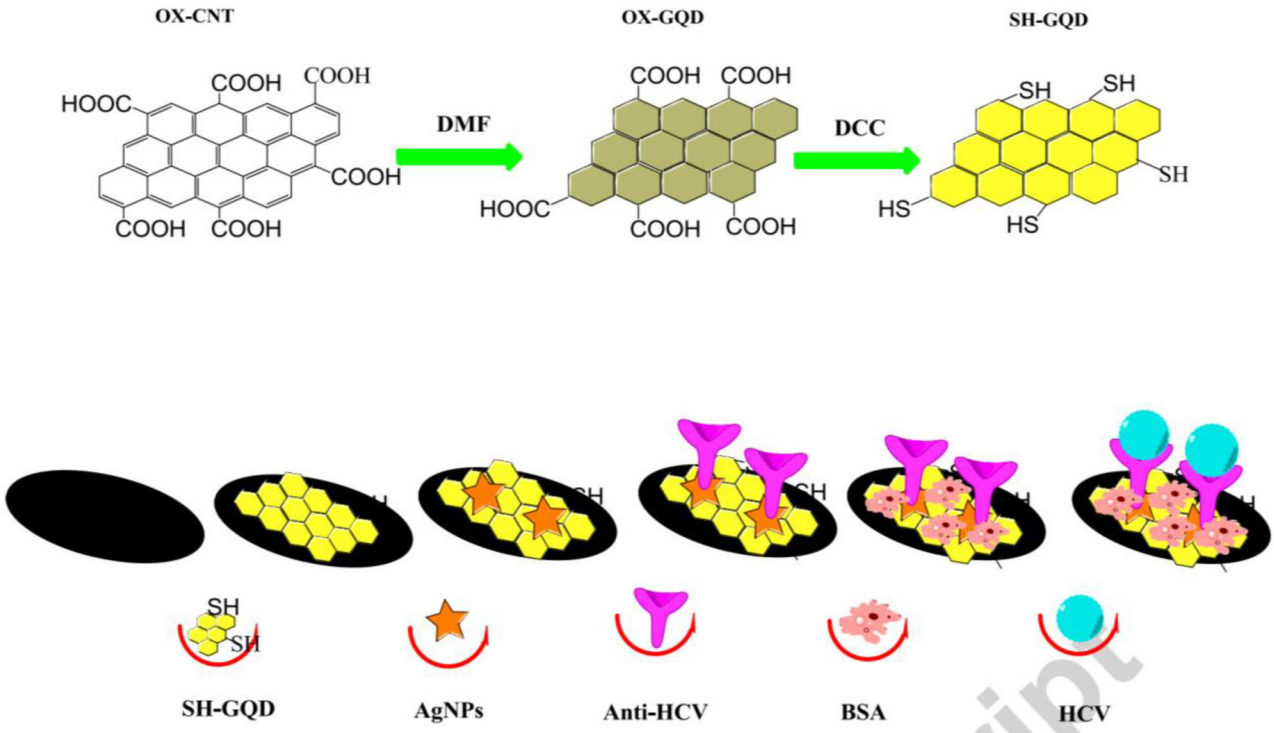



**Figure 16.** Schematic illustration of the proposed electrochemical immunosensor for hepatitis C virus core antigen (HCV) detection. OX:CNT: oxidated carbon nanotubes, OX:GQD: oxidated graphene quantum dot, AgNPs: silver nanoparticles, GQD-SH: thiol graphene quantum dots. Reprinted from Ref. [104] with permission from Elsevier.

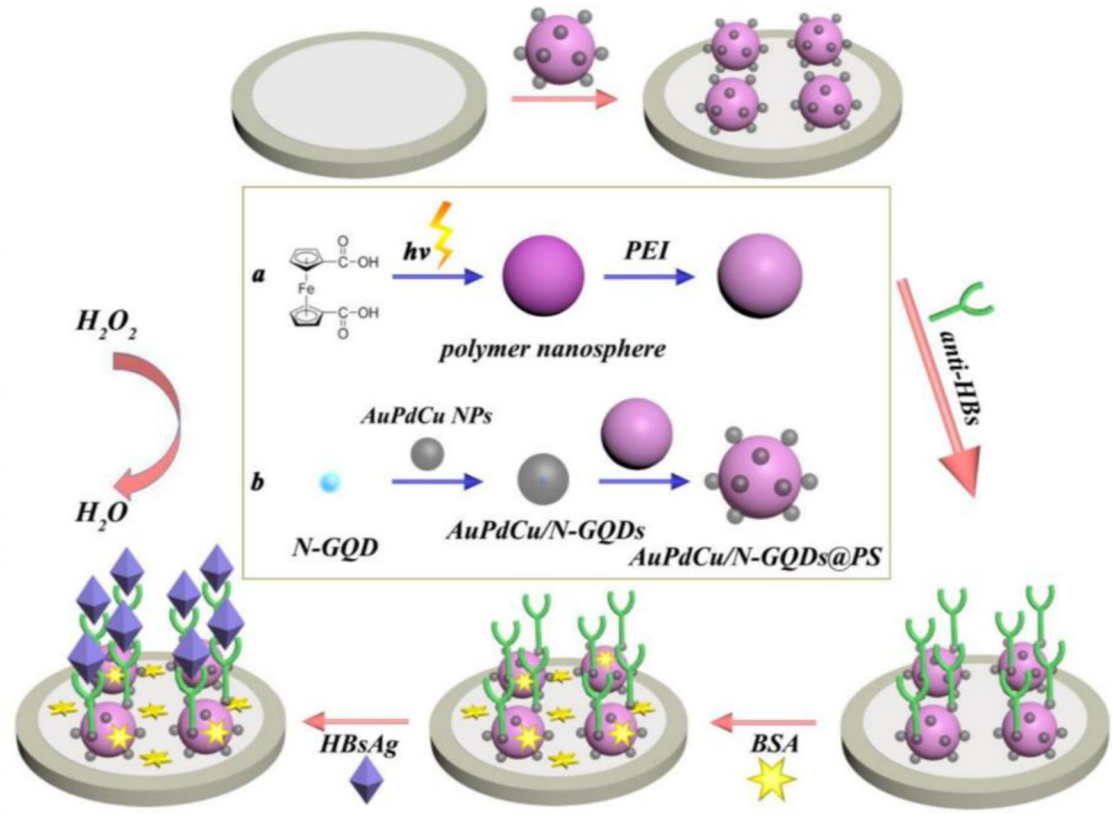



**Figure 17.** The schematic illustration of the label-free electrochemical immunosensor. The preparation procedure of the polymer nanospheres (PS) functionalized with Polyethylenimine (PEI) (a), and the fabrication procedure of AuPdCu gold/palladium/copper ternary nanoparticles with nitrogen doped graphene quantum dots on PS AuPdCu/N-GQDs@PS (b). HBsAg: hepatitis B surface antigen. Reprinted from Ref. [105]

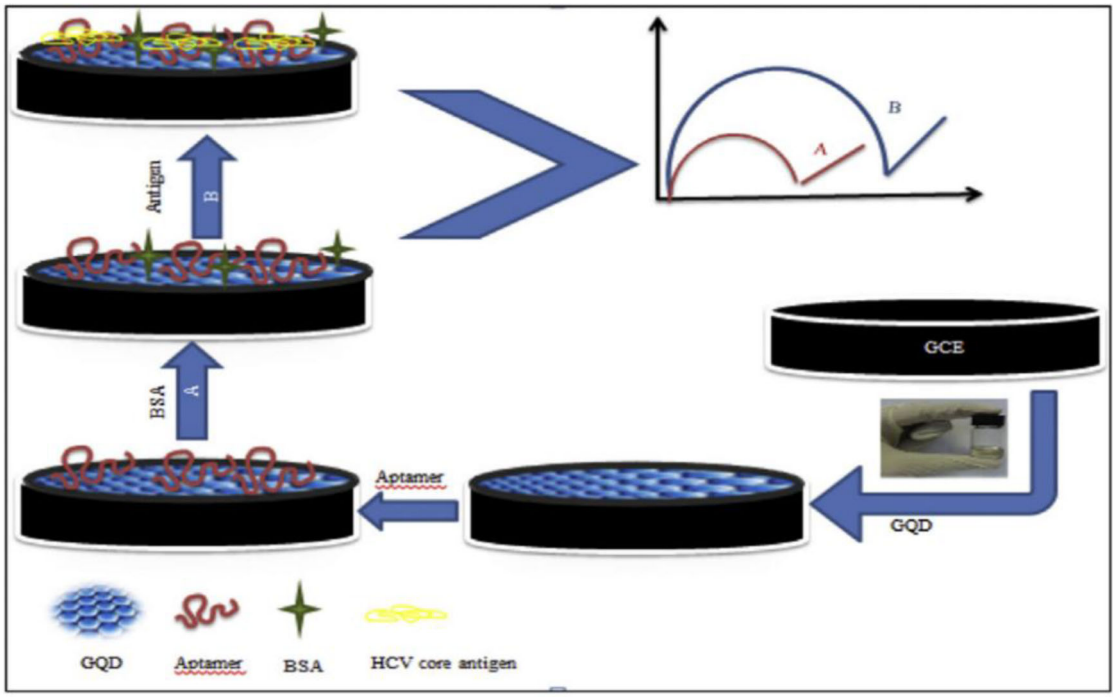



**Figure 18.** Schematic representation of electrochemical nano aptasensor for HCV core antigen detection. Reprinted from Ref. [106] with permission from Elsevier.

reduce LOD levels in early viral sensing because of the wealth of hydrophilic edges as well as of the hydrophobic plane in GQD, which increases the aptamer adsorption on the electrode surface. Ghanbari et al. have developed an electrochemical aptasensor for sensitive detection of hepatitis C virus (HCV) core antigen by using the aptamer proximity binding assay strategy.[106] A schematic representation of the sensing principle is shown in Figure 18. The immobilization surface is prepared by modifying a glassy carbon electrode (GCE) with graphene quantum dots (GQD). EIS technique was successfully performed as an efficient detection system for HCV core antigen measurement in two linear concentration range 10–70 pg $mL^{-1}$ and 70–400 pg $mL^{-1}$ with a detection limit value of 3.3 pg $mL^{-1}$. Moreover, the developed aptasensor could accurately detect HCV core antigen concentration in human serum samples.

Electrochemical DNA sensors have garnered significant interest from researchers, since they play a vital part in the early diagnosis of infectious illnesses as an interesting and crucial application in biomedical science research.[95] Xiang et al. have created, by use of the GQD-modified graphene quantum dot and special sequence of DNA molecules, a simple, smart electrochemical platform for identifying HBV-DNA, based on a strong interaction between the single-stranded DNA and GQD.[107] A schematic representation of the sensing principle is shown in Figure 19. In this system, the probe DNA is designed to be complementary to the HBV-DNA. When the probe DNA is strongly bound to the surface of the GQD modified electrode, the electron transfer from the electrode to the redox species will become problematic. In the presence of the target HBV-DNA in the test solution, the probe DNA can bind to the target HBV-DNA instead of GQDs. As a result, the electrochemical signal increase with the different concentrations of the target

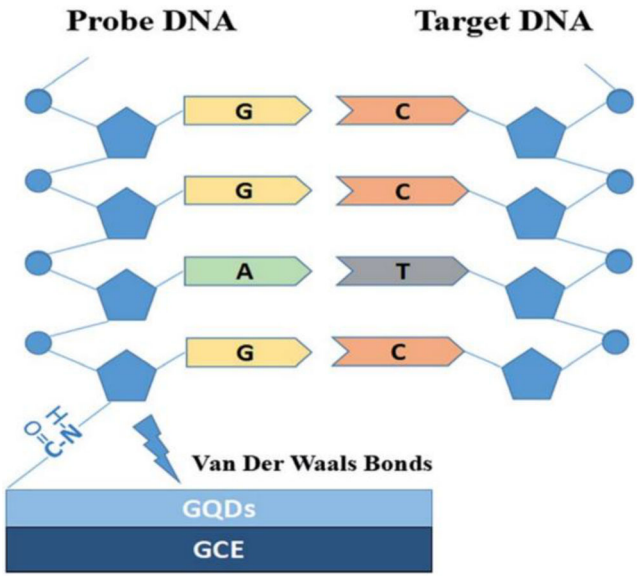



**Figure 19.** Schematic representation of electrode preparation for DNA detection. Reprinted from Ref. [107] with permission from Royal Society of Chemistry.

HBV-DNA. In particular, the proposed sensor showed high sensitivity in the linear detection range between 10 nM and 500 nM with a detection limit of 1 nM.

In another study based on a similar approach in the preceding study, Salimian et. al. offered a sensitive and simple viral sensing method through electrochemical detection of DNA based on hollow carbon spheres (HCS) decorated polyaniline (PANI).[108] A thiolated 21-mer oligonucleotide, characteristic of HBV DNA was immobilized via electrodeposited gold nanoparticles on HCS-PANI. A schematic representation of the sensing principle is shown in Figure 20. At a concentration as low as 10 fM, the proposed biosensor can detect the target sequences of HBV DNA. Furthermore,

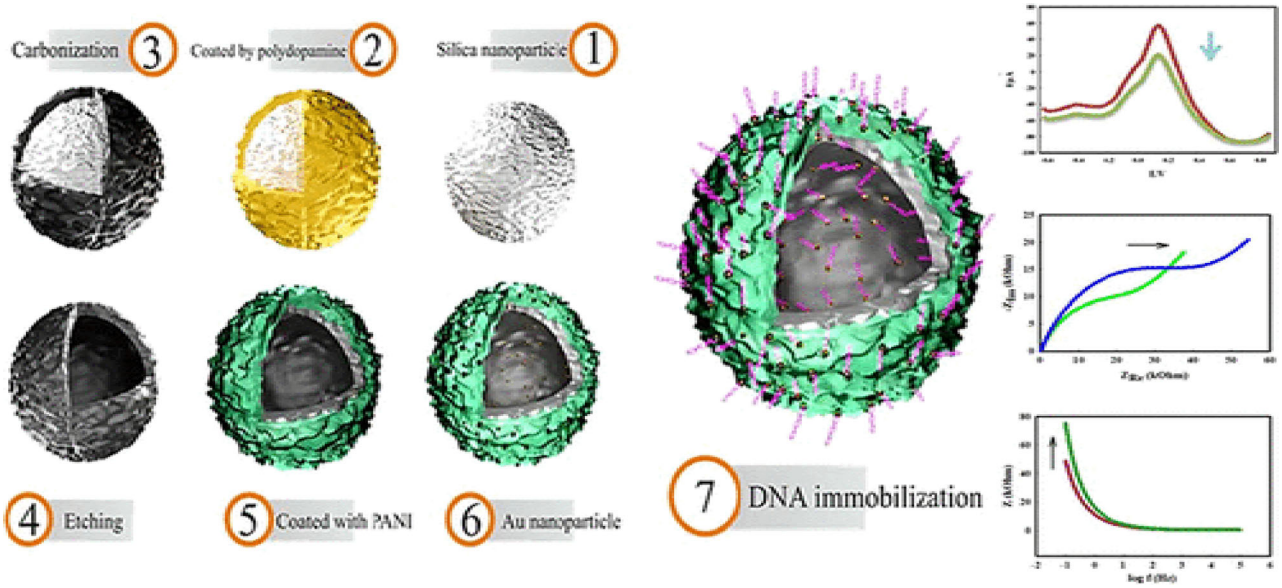



Figure 20. Schematic representation of material preparation and electrochemical sensing processes. Reprinted from Ref. [108] with permission from ACS.

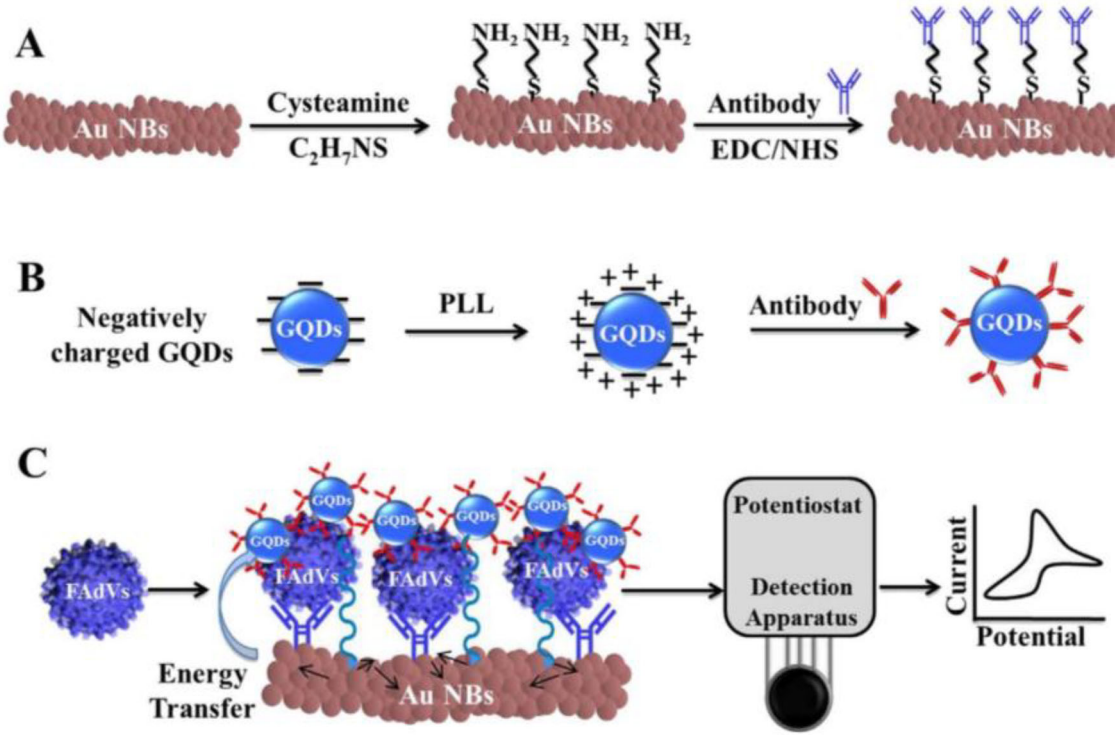



Figure 21. Schematic presentation of fowl adenovirus (FAdVs) sensing: The gold nanobundles (Au NBs) (A) and graphene quantum dots (GQDs) (B) were first conjugated with anti-adenovirus antibodies. Then the anti-adenovirus conjugated Au NBs and GQDs to form a complex (C) in the presence of target FAdVs, finally enhancing the current peak due to light-matter interaction. Reprinted from Ref. [109] with permission from Elsevier.

the suggested biosensor was able to distinguish between a completely matched target oligonucleotide and three nucleotide mismatched oligonucleotides. Moreover, this electrochemical DNA sensor detected HBV DNA in actual samples with high sensitivity.

An optoelectronic sensor for practical applications is a fast diagnostic tool, which enables an exact, dependable, field-portable, and low-cost instrument. Ahmed et al. developed an optoelectronic sensor for the detection of fowl adenoviruses (FAdVs) based on free gold nanobundles (Au NBs).[109] The surface of carbon electrodes was modified by using L(+) ascorbic acid, gold chloroauric acid and poly-L-lysine (PLL) via layer-by-layer (LbL) approach. GQDs have been produced using an autoclave-aided technique in this work. Both GQDs and Au NBs were conjugated with target FAdVs specific antibodies that bring them nearby using antibody-antigen interaction to add target FAdVs. Nearby the light-matter interaction between Au NB and QD generates a local electric signal improvement by UV-visible irradiation, which permits the detection of extremely low levels of target virus in complicated biological matrices. Figure 21 shows a schematic depiction of the sensing concept. The

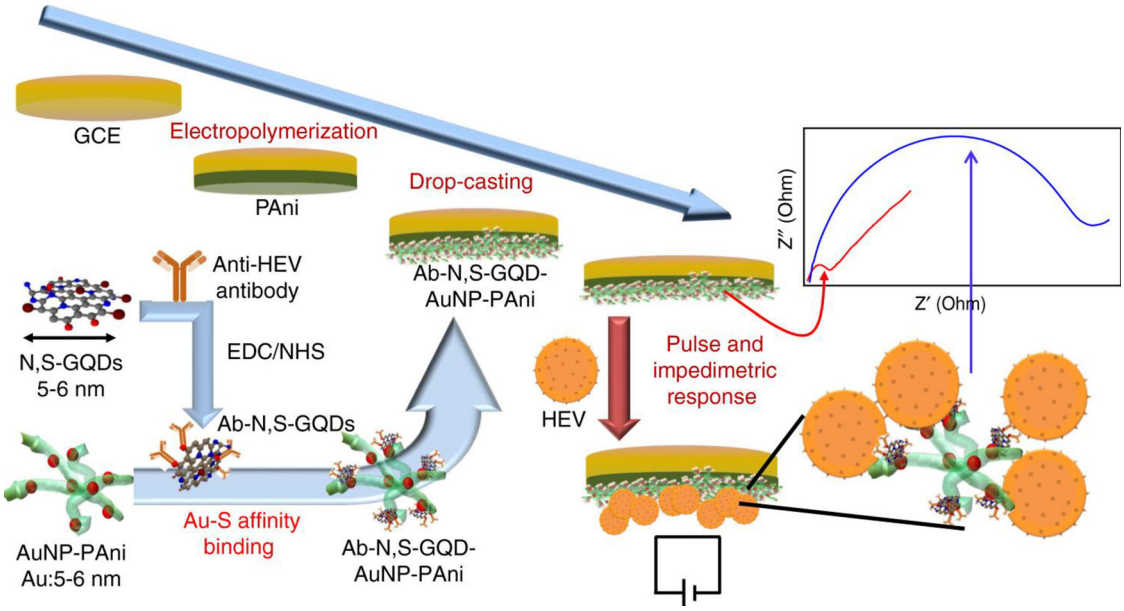



**Figure 22.** Schematic diagram of the antibody functionalized nitrogen and sulfur doped-graphene quantum dots on gold nanoparticles-polyaniline (Ab-N,S-GQDs@AuNP-PAni) nanocomposite-loaded sensor electrode and its pulse-induced impedimetric sensing of hepatitis E virus (HEV). Reprinted from Ref. [110] with permission from Nature Publishing Group.

proposed optoelectronic sensor showed a linear relationship (10 PFU/mL to 10,000 PFU/mL) between the target FAdVs and the electric signal up to 10 Plaque forming units (PFU)/mL with a limit of detection (LOD) value of 8.75 PFU/mL. The proposed sensing approach was found 100 times more sensitive than the conventional ELISA method.

One of the major causes of acute viral hepatitis virus in the globe is hepatitis E virus (HEV).[110] The pulse triggered ultrasensitive electrochemical sensor was made by Chowdhury et al. utilizing graphene quantum and gold embedded polyaniline nanowires, produced via a polymerization interface and afterwards self-assembly process. N,S-GQDs were prepared by hydrothermal method with thiourea and citric acid. Introducing an external electrical pulse during the virus accumulation step increases the sensitivity toward HEV due to the expanded surface of the virus particle as well as the antibody-conjugated polyaniline chain length compared to other conventional electrochemical sensors. The sensor was evaluated on several HEV genotypes, including G1, G3, G7, and HEV ferrets acquired from the supernatant cell culture and collected from the G7-HEV infected monkey in many fecal specimen samples. The sensitivity is similar to that by the real-time quantitative reverse transcription-polymerase chain (RT-qPCR). This sensor can pave the way for the development of robust, high-performance HEV detection sensing methods. A schematic representation of the sensing principle is shown in Figure 22.

Table 3 shows the summary of different approaches for CDs synthesis and electrochemical detection of viruses. It is seen that primarily preferred synthesis methods are pyrolysis and hydrothermal synthesis, and the most used precursor is citric acid. It is also important to remark that mostly proteins, specific antigens, or DNA viruses are the target analyte. Therefore, DNA probes to target DNA and specific antibodies to proteins are widely used as recognition units in designed sensing principles. In addition, it was observed that all these recently reported studies provided promising detection limits and exhibited outstanding sensor performance.

## Conclusion and future perspectives

Many of the infectious disease diagnostic techniques used today need a considerable time, a laboratory setting, and skilled staff. In many countries, people do not live with local access to health care. It is frequently not possible for them to enter a biological sample on two different days and to collect findings and start therapy for a second time. Taking all this into account and taking into account the evolution of viruses into more strong forms and antibiotic resistance in microbes, humans need to be better prepared to identify harmful species in the future by creating easy-to-use detection technologies.

Since the introduction of smartphones and other mobile devices, scientists have been paying great interest in wearable sensors, because they can give vital insights into individuals' health. Many indicators (including protein and nucleic acids, pathogenic species) will eventually be monitored in an intrusive fashion by future wearables, thereby providing a full medical and performance evaluation. From a perspective, wearable biosensors are predicted to be simplified and move away from the wrist into fabrics and fashion items that fuze into the daily life of the wearer. Furthermore, several of these gadgets will require disposable components to address fouling issues. The combination of CDs' features, such as high fluorescence, electrical conductivity, and biocompatibility with wearable sensing devices can pioneer new advances in direct pathogen detection, which is crucial for early detection, appropriate treatment, and control of the transmission.

Immediately following an invasion of foreign material, antibodies generated may report initial infections,

**Table 3.** Studies on carbon-based nanodots for electrochemical detection of viruses.

| Carbon-based fluorecent material | Precursors | Synthesis | Quantum Yield | Target Virus | Target Analyte | Recognition Matrix | Linear Range | LOD | Application | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Graphene quantum dots | Graphene oxide | Microwave irradiation | — | ALVs-J | ALVs-J | ALVs-J antibodies | $10^{2.08}$–104.50 $TCID_{50}$/mL | 115 $TCID_{50}$/mL | Electrolyte solution | [103] |
| Thiol graphene quantum dots (GQD-SH) | MWCNTs | Oxidative cutting | — | HCV | HCV core antigen | Anti-HCV core antibody | 0.05 pg/mL–60 ng /mL | 3 fg/mL | PBS solution pH 7.0 and | [104] |
| Nitrogen-doped graphene quantum dots | Citric acid and dicyandiamide | Hydrothermal method | — | HBV | HBV surface antigen | Anti-HBV surface antibody | 10 fg/mL–50 ng/mL | 3.3 fg/mL | Human serum | [105] |
| Graphene quantum dots | Citric acid | Pyrolysis | — | HCV | HCV core antigen | Core-specific aptamer | Two linear concentration ranges: 10–70 pg/mL 70–400 pg/mL | 3.3 pg/mL | Human serum | [106] |
| Graphene quantum dots | Citric acid | Pyrolysis | — | HBV | HBV-DNA | DNA probe | 10 nM–500 nM | 1 nM | Electrolyte solution | [107] |
| Graphene quantum dots | *L*(+)-ascorbic acid and graphene nanopowder | Hydrothermal method | 11.26% | FAdVs | FAdV-9 | FAdVs specific antibodies | 10 PFU/mL to 10000 PFU/mL | 8.75 PFU/mL | Chicken blood | [109] |
| Nitrogen- and sulfur-codoped graphene quantum dots | Thiourea to citric acid | Hydrothermal method | — | HEV | HEV like particles and HEV | Specific anti-HEV antibody-conjugated | $10^{2}$–$10^{7}$ RNA copies/L | 96.7 RNA copies/L | Fecal specimen samples collected from G7 HEV-infected monkey | [110] |
| Hollow carbon spheres | Dopamine hydrochloride | Pyrolysis | — | HBV | HBV-DNA | DNA probe | 0.01 pM–1 nM | 3.62 fM | Diluted human blood plasma | [108] |

Abbreviations: ALVs-J: avian leukosis virus subgroup J (ALVs-J); HCV: hepatitis C virus core antigen; MWCNTs: multiwalled carbon nanotubes; HBV: hepatitis B; DNA: deoxyribonucleic acid; FAdVs: fowl adenoviruses; FAdV-9: Fowl adenovirus-9 virus; PFU: Plaque forming unit; HEV: Hepatitis E virus.

reinfections, or reactivation status. There is, therefore, a well-accepted method for detecting pathogenic conditions for measuring immunoglobulin (Ig) levels. In this sense, CDs can serve as an emerging instrument to attain a lower detection limit using small sample volume equipment, such as microfluidic paper/plastic-based assays, for early diagnosis. In addition, pathogen detection by combining the unique properties of CDs and advantages CRISPR approach could result in sensitive and early detection assay production.

Given the competition and enormous trade prospects for pathogen detection, there will soon be interesting new advancements. To this end, future tendencies and challenges to overcome are; (i) the development of new classes of sensing tools with "green" materials used for sustainable, biodegradable, and inexpensive production; (ii) the miniaturization and use of portable devices (such as smart portable devices); (iii) implementation of entirely integrated, disposable sensor technology, comprising readout elements and electricity source (for instance batteries), use of signal enhancing functional nanomaterials.

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