Carbon Dot-Based Biosensors

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0D fluorescent carbon dots (CDs), consisting of $sp^2/sp^3$ carbon skeletons with functional groups, have the advantages of good chemical stability, high biocompatibility, excellent dispersibility in water, and low photobleaching, as well as low toxicity and low cost. These interesting features allow CDs to be applicable in the field of biological imaging, optoelectronic, and energy devices, especially in the application of biosensors, which are rapidly gaining attention. Herein, CD synthesis methods are summarized in detail, and the basic scientific issues of top-down and bottom-up approaches to CDs photoluminescence mechanisms are comprehensively discussed and analyzed. Then, valuable insights into the current status of CD research for biosensor applications are provided and the basic photoluminescence properties of CDs, focusing on the CD photoluminescence mechanism from the aspects of optical absorption, up-conversion glow, static and dynamic quenching, fluorescence resonance energy transfer (FRET) quenching, photoinduced electron transfer (PET) quenching, and inner filter effect (IFE) quenching, are explained. Finally, a brief outlook is given to stimulate new ideas and promote further research on the potential use of CDs in various biosensor fields.

1. Introduction

Carbon, as one of the very common elements, is widely distributed in the atmosphere and Earth’s crust and in life in its many forms, which is also the key attribute for promoting the development of this element in industry and society. As we all know, graphite, fullerenes, carbon nanotubes (CNTs), and graphene are important carbon family members. In recent years, a new type of carbon, namely luminescent carbon nanomaterials, has received widespread notice and has gradually become a research focus. 0D fluorescent carbon nanomaterials with specifications below 10 nm are generally called “carbon dots” (CDs), consisting of an $sp^2/sp^3$ carbon skeleton with many functional groups. The development of CDs can be traced back to Xu et al.’s report on fluorescent single-walled carbon nanotubes (SWNTs). According to the principle of electrophoresis, two new nanocomposite materials were separated from the crude fragrant ash, including short tubular carbon and fluorescent nanoparticles derived from SWNTs. Bright and colorful photoluminescent (PL) CDs were synthesized in large quantities. Compared with traditional organic dyes or bright inorganic quantum dots (QDs) technology, CDs have the advantages of good organic chemistry reliability, high compatibility, excellent dispersibility in water, and low cost, as well as low toxicity and photobleaching. These interesting features allow CDs to be applicable in the field of biosensors, bioimaging, optoelectronic devices, and energy-related applications.

As summarized from the Web of Science database in Figure 1A, research on CDs has been increasing steadily since 2012, indicating that the high expectations of CDs have received growing interest from researchers. In particular, research focusing on CD-based biosensors, including optosensors, electrochemical and electrochemiluminescence (ECL) biosensors, photoelectrochemical (PEC) biosensors, quartz crystal microbalance (QCM)-based biosensors, surface plasmon resonance (SPR)-based biosensors, resonance Rayleigh scattering spectroscopy/surface-enhanced Raman spectroscopy (SERS)-based biosensors, and thermal biosensors (Figure 1B), has attracted attention. Our group and others have developed CD-based biosensors for a range of DNA, proteins, enzymes, ions, molecules, bacteria, cells, and so on (Figure 1C).

With the focus on the bioanalytical aspects of CDs, a lot of related literature has been published recently. CDs are chemically inert and optically stable, making them very suitable for nanoprobe for optical biosensor development. We will provide an update on the latest research and systematically summarize CD applications in the field of biosensors in this Review. In Section 2, we will briefly introduce CDs fabrication. We will focus on many basic scientific issues of top-down and bottom-up methods for CD photoluminescence mechanisms. Section 3 covers the latest and advanced applications of CDs in the field of biosensors. In Section 4, we will explain the basic photoluminescence properties of CDs, focusing on the CDs photoluminescence mechanism from the aspects of optical absorption, up-conversion glow, static and dynamic quenching, fluorescence resonance energy transfer (FRET) quenching.
photoinduced electron transfer (PET) quenching, and inner filter effect (IFE) quenching. Finally, we present a perspective for CD-based biosensors, including potential applications and possible development trends.

2. Fabrication of CDs

Over the past decade, the preparation of CDs has attracted wide attention. Based on the changing direction of the particle size during the preparation process, the synthesis of CDs can be summarized as top-down and bottom-up syntheses. The top-down method breaks down or splits larger carbon structures into smaller nanoscale carbon structures through physical, chemical, or electrochemical methods. For example, the size of materials with perfect $sp^2$ conjugate structures, such as graphite, graphene, or CNTs, can be reduced. The bottom-up method generates carbon nanoparticles (CNPs) by pyrolysis and carbonization of small organic molecules or polymerization with a small size range. This method can control the structure, shape, and size of CDs by designing precursors and preparing processes.

2.1. Top-Down Method

2.1.1. Arc Discharge Method

The preparation of carbon nanomaterials has attracted widespread attention in the past decade. An electrophoretic purification method for preparing SWCNTs using arc discharge soot was first reported in 2004. During the experiment, three types of materials were obtained by separating the suspension through gel electrophoresis, including long nanotubes, short and irregular tubular materials (Figure 2A), and highly fluorescent materials. Centrifugal ultrafiltration tube filtration equipment was used to separate the highly fluorescent materials to obtain three fluorescent materials with different average molecular weights. They emitted blue-green, yellow, and orange fluorescence under 365 nm excitation light, and the fluorescence quantum yield (QY) of the yellow fluorescent material measured under 366 nm excitation was 0.016. Bottini et al. used original SWCNTs and CNTs oxidized by nitric acid as carbon sources and prepared fluorescent nanoparticles using arc discharge method to obtain fluorescence from blue-violet and blue to light yellowish–green nanoparticles, whose fluorescence peak gradually red shifted as the molecular weight of the fluorescent nanoparticle.

Figure 1. A) Annual publications on CDs and biosensors based on CDs in Web of Science and timeline, where CD synthesis was first reported. B) Number of articles and events based on CD biosensors. C) Application of CDs in sensors.
increased; the photoluminescence of the fluorescent nanoparticle showed a molecular weight dependence.\textsuperscript{[25]} Dey et al. synthesized boron-doped graphene QDs (B-GQDs) and nitrogen-doped GQDs (N-GQDs) by a gas-phase arc discharge method, which improved the shortcoming of the small bandgap of graphene. Under visible light excitation, up-converted PL emission can be observed in B-GQDs, N-GQDs, and undoped GQDs.\textsuperscript{[26]} As the earliest method of preparing CDs, the advantages of electric solitary charging and discharging are the small particle size of CD and high oxygen content. The disadvantage is that there are more impurities, and the CD yield and fluorescence QY are low.

2.1.2. Laser Ablation Method

The laser ablation method abrades a carbon target with a laser at high temperature and pressure to obtain CNPs, which are then modified and surface functionalized to obtain CDs. Sun et al. used laser ablation to produce CDs under the condition of using argon as a water vapor carrier gas. These samples and their aqueous suspensions showed no detectable photoluminescence, and surface passivation by poly-(propionylethylene-imine-co-ethyleneimine) (PPEI-EI) produced bright fluorescence (Figure 3B).\textsuperscript{[3]} Mechanistically, the photoluminescence produced
by CDs may be due to surface passivation that causes surface energy traps to become emissive upon stabilization. Sun and his colleagues found that CNPs can be doped with inorganic salts such as ZnO or ZnS prior to passivation of the surface of organic molecules to obtain higher photoluminescence QY,[27] which reached values above 50%. Hu et al. reported a simple one-step synthesis method, in which graphite powder was dispersed in three solvents, such as diethanolamine, diamine hydrate, and poly(ethylene glycol) (PEG200N). The results showed that the carbon materials had a negligible effect on the optical properties of the CDs, whereas solvents can effectively change the color and intensity of light. In this method, carboxyl radicals showed the surface conditions of the photoluminescence of the CDs,[28] and the formation of CDs and their surface modification are completed simultaneously. A straightforward synthesis process of CDs by ultraviolet (UV)-pulsed laser-irradiating carbon targets in water and their functionalized reaction with NH₂-PEG200 and N-acetyl-l-cysteine (NAC) were reported.[29] Bagga et al. reported on a high-purity graphite target in deionized water, which was ablated with a Nd:YAG laser software (WEDGE HF 1064, Bright Solutions, 1064 nm, 10 kHz) to prepare high-quality CNPs with controllable viscosity and high water stability. The CD surface showed sufficient electrostatic stability to avoid CD agglomeration or flocculation.[30] Sidorov et al. used the technical application of a nanosecond near-infrared spectrometer-pulsed light burnout of amorphous carbon film to produce CD burnout zone of nanodiamonds (NDs) with a graphite structure and a velocity surface layer on the mattress layer tightly surrounded. The characteristic specifications of CDs and NDs were 20–300 nm and 30–500 nm, respectively. The NDs were mainly formed around the laser torch at the boundaries of the reorganized plasma area, whereas the CDs were formed directly in the dissolution area and around the expanded area of the reconstituted plasma.[31] Although this method is simple and can obtain all kinds of nanostructures, it requires more carbon materials, the preparation process is more complicated, the equipment is more expensive, the yield of the synthesized CDs is lower, and the particle size of the CDs is not uniform. These shortcomings have to be addressed.

Figure 3. A) TEM image and spectrum of CDs@zeolite composites. Reproduced with permission.[47] Copyright 2019, American Chemical Society, some rights reserved. B) GQD composite photocatalytic process and spectrogram. Reproduced with permission.[50] Copyright 2012, American Chemical Society. C) N-CDs and spectrogram. Reproduced with permission.[55] Copyright 2015, The Royal Society of Chemistry. D) CDs and UV spectrogram. Reproduced with permission.[64] Copyright 2014, The Royal Society of Chemistry.
2.1.3. Electrochemical Synthesis Method

The electrochemical method is a method of preparing CDs by repeatedly charging and discharging a carbon source as a working electrode. Zhou et al. first reported the preparation of CDs by electrochemical synthesis. The research group used CNTs as a carbon source and prepared CDs by adding multiwalled carbon nanotubes (MWCNTs) to an acetonitrile solution containing 0.1 M tetrabutylammonium perchlorate (TBAP). The CDs produced bright blue fluorescence. Li et al. reported water-soluble CDs cut from graphite rods under alkaline conditions, which could be size controlled, and showed excellent fluorescent properties. However, the loss of graphite rods after electrochemical treatment was inconsistent with the quality of CDs prepared; the amount of fluorescent carbon products was greater than the loss of graphite. The research group studied and indicated that these additional carbon products were derived from ethanol. At the same time, a method for preparing fluorescent CNPs by electrochemical treatment of ethanol with subsequent sodium hydroxide treatment was reported. A strategy for producing light-emitting CDs by electrochemically etching carbon fibers in a controlled manner has been proposed by Bao and his colleagues. Synthetic control of monodisperse luminescent CDs can be achieved by adjusting the applied potentials without surface passivation or further separations. At the same time, the CD size can be increased by extending the electrochemical reaction time during preparation. Ming et al. used graphite as a carbon source to synthesize CDs in a one-step electrochemical method, and most of the products obtained were multilayer graphene oxide (GO). The as-obtained CDs featured remarkable down- and up-converted PL properties and can improve the catalytic and photovoltaic properties of TiO₂ through bonding and interaction with TiO₂ (Figure 3C). The electrochemical method can give rise to uniform CDs by adjusting the current density and electrode potential. The utilization rate of carbon sources is relatively high, and the cost is low. Despite the required time-consuming pretreatment and postpurification of the raw materials, some CDs do not need further purification.

2.1.4. Combustion Method

The combustion method generally uses simple materials, such as candle ash, natural gas ash, and paraffin putty, which are burnt to prepare CDs. Liu et al. first reported the preparation of multicolor fluorescent CNPs using the combustion soot of a candle under reflux in nitric acid (Figure 3A). Subsequently, many researchers, such as Ray et al. and Tao et al., prepared fluorescent CDs by collecting ash from burning candles and straws, followed by oxidation with nitric acid. The CDs prepared by the combustion method can emit light without surface passivation, and the CD particles are relatively uniform. However, this method generally requires oxidizing acid treatment, which affects the fluorescence properties and produces CDs with low-fluorescence QY.

2.1.5. Chemical Oxidation Method

Compared with other methods, the preparation of CDs by the chemical oxidation method is less frequent. It is a method of oxidizing and cutting carbon materials into CDs with a strong oxidant. Peng et al. added a pitch-based carbon fiber to a mixture of concentrated sulfuric acid and concentrated nitric acid and treated it to obtain good crystallinity. Via acid peeling and etching of the pitch carbon fibers, abundant distributed graphite domains were found in its original skeleton, and QDs were synthesized on a massive scale. Zhou et al. mixed and stirred a GO suspension, 30% hydrogen peroxide (H₂O₂), and ferric chloride (FeCl₃·6H₂O) solution and irradiated it with a mercury lamp to prepare fluorescent CDs with good crystallinity. In recent years, an improved nitric acid oxidation method for preparing CDs from industrial W45 carbon black (WCB) was reported and CDs with dual-tunable luminescence characteristics were obtained, which were closely related to the effects of the oxygen doping process (Figure 4D). This method offers the advantages of low cost and small particle size of the CDs, and the product may have different colors of light, but the synthesis process is complicated, which is not beneficial for product collection.

2.2. Bottom-Up Method

2.2.1. Solvothermal Method

Solvothermal synthesis is one of the important methods of CD synthesis, which involves a direct solvothermal reaction under high temperature and high pressure in a reactor. Liu et al. first demonstrated a heat treatment-based strategy to prepare PL CDs by polymerization of CCl₄ and 1,2-ethylenediamine (EDA) under reflux, microwave, or solvothermal heating. Li et al. proposed a new one-step hydrothermal method for the surface passivation of nitrogen-doped CDs (N-CDs) with linear-structured poly(ethyleneimine) (PEI) as reagent/nitrogen source and citric acid as raw material. Due to the effects of N doping, surface passivation, and size, the N-CDs showed unique down-conversion photoluminescence and a high spectral QY of 37.4%. Jiang et al. directly used p-phenylenediamine as a synthetic precursor of CDs and prepared PL CDs with a maximum emission peak of 620 nm. The three resulting CDs emitted bright and stable red, green, and blue (RGB) light after a single UV light excitation. It is believed that the changes in photoluminescence emission are due to the differences in the particle sizes and nitrogen content. In recent years, Li et al. successfully used ginkgo leaves as a CD source to prepare nitrogen- and sulfur-codoped CDs (N- and S-CDs). The fluorescence intensity of the N- and S-CDs was quite stable at different pH values and ionic strengths. Wang et al. proposed a simple strategy for regulating the donor–acceptor energy transfer (EnT) of CDs in molecular sieves to regulate the red-emitting room-temperature phosphorescence (RTP) properties of the CDs; they prepared CDs@zeolite (CDs synthesized by the author) composites with green and red RTP using in situ hydrothermal synthesis by adjusting the doping amount of heteroatoms (Zn²⁺ and Mn²⁺) in the framework (Figure 4A). The zeolite matrix was effective in stabilizing the triplet state of the CDs. The solvothermal synthesis method has the characteristics of simple operation, easy control, high yield, and a wide range of raw reaction materials and is suitable for industrial production. However, the disadvantage of this method is the difficulty to control the particle size of CDs, which requires the use of organic toxic solvents. However, the reaction
is conducted in a closed system, which can effectively block the volatilization of toxic substances, so it is relatively friendly to the environment.

2.2.2. Ultrasonic Method

The ultrasonic method uses activated carbon, glucose, etc. as carbon sources, and ultrasonic-assisted decomposition of the carbon source materials is used to prepare CDs in presence of strong acids or bases. Li et al. directly synthesized monodispersed water-soluble fluorescent CNPs using glucose as raw material by a one-step alkali- or acid-assisted ultrasonic treatment. Subsequently, the research group directly synthesized water-soluble fluorescent CNPs using activated carbon as the raw material in the presence of hydrogen peroxide. CNPs emitted bright and colorful photoluminescence, belonging to the category of near-infrared spectroscopy. In addition, CNPs also showed excellent up-conversion fluorescence characteristics. N-CDs were also synthesized. Zhuo et al. presented a convenient ultrasonic route to prepared GQDs with up-converted emission (Figure 2B). The prepared GQDs mainly exhibit PL individual behaviors that are irrelevant to arousing down-conversion and up-conversion. Zhu et al. created a one-step ultrasound method for synthesizing GQDs. Ascorbic acid and other materials can be used as carbon sources for synthesizing CDs. The ultrasonic method is simple to operate, but the biggest disadvantage is the low yield of CDs.

2.2.3. Solid-Phase Synthesis Method

Solid-phase synthesis methods are rarely used in practical applications. A simple bottom-up preparation of blue PL GQDs and GO was developed in 2012 by adjusting the degree of carbonization of citric acid and dispersing the carbonized products in alkaline solutions, and the color change of the liquid to orange during synthesis indicated the formation of CDs. More interestingly, Ruan et al. used glycine as the only precursor and prepared CDs, which can be used for in vivo experiments. Chen et al. designed a method to prepare N-CDs by immediately heating the solid compound of folic acid (FA) tablets and sodium citrate (SC) to 300 °C. The total number of N doping content not only jeopardizes the transmission QY, but also jeopardizes the wavelength of the transmitted light. According to the excitation wavelength and the amount of N doping, due to their good water solubility, strong blue–green fluorescence and low toxicity, (Figure 2C), and resistance to 15 types of photobleaching,
N-CDs can be taken up by multiple cells and serve as an ideal candidate for multicolor cell imaging. Therefore, heteroatom-doped CDs may have more suitable properties than nondoped CDs. Common doped CDs are N- and S-CDs. Generally, in the solid-phase synthesis method, a carbon source material with a structure similar to that of CDs should be selected, but during the actual operation, the temperature is not easy to control, and the luminescence performance of the obtained CDs is unstable.

2.2.4. Microwave Pyrolysis Approach

In recent years, the use of microwave methods has become more frequent. Zhu et al. synthesized a simple and economically developed microwave heating pyrolysis method to generate fluorescent CNPs. Next, the research group developed a green and economical method for the one-step microwave synthesis of multicolor fluorescent CDs without the need for surface passivation reagents. Liu et al. used cheap glycerol as the raw material and used 4,7,10-trioxa-1,13-tridecanediamine (TTDDA) as a surface passivation agent when generating CDs and introduced a simple, fast, and low-cost method in detail for microwave-assisted pyrolysis of carbohydrates to build CDs with strong photoluminescence properties. A one-step microwave heating-assisted polyether polyl method was developed and designed by Liu et al., using sucrose as the carbon source and diethylene glycol (DEG) as the reflecting substance to prepare the emerald green and shiny CDs. Gong et al. and Guan et al. conducted a similar experiment. The greater advantage of the one-step method is that it can additionally complete the generation of CNPs and the surface passivation treatment. Wang et al. reported a microwave pyrolysis method for the large-scale synthesis of strong luminescent CDs using sulfuric acid as catalyst and benzenediols (catechol, hydroquinone, and resorcinol) as precursors (Figure 3D). Dhenadhayalan et al. used methanol as raw material, and Liu et al. used glutaraldehyde (25% in H₂O) and PEI as raw materials. Each of these studies prepared unique luminescent CDs. The microwave method benefits from a simple design. The excellent penetration ability of microwaves results in a fast reaction. Generally, it is a very convenient method for preparing light-emitting CDs.

2.2.5. Template Method

Next, we will introduce the template method for preparing CDs. A straightforward and novel method for amorphous nanosized (1.5–2.5 nm) CDs was reported in 2009; among them, a silica ball of surfactant-modified material was used as the medium and the fusible urea–formaldehyde resin was used as the carbon precursor system to prepare the CDs (Figure 4B). The results showed that the QY of PEG₁₅₀₀₀ (polyethylene glycol), H₂NCH₂(CH₂CH₂O)nCH₂CH₂CH₂NH₂-passivated CDs, was 14.7%, which was considered very high at that time. Kwon et al. used a soft-template method to synthesize CDs, which can be used as novel photoactive materials, with citric acid as a precursor. Water droplets containing precursor molecules were stabilized with emulsifiers (oleylamine). Heating led to the evaporation of water, which in turn promotes the dehydration of the molecular structure of SC, resulting in a “polymer-like” chemical intermediate. This chemical intermediate is carbonized and additionally oiled. Covered with amine molecules, organosoluble CDs were obtained. The specification of CDs can be manipulated by adjusting the amount of emulsifier. With the increase in size, the CDs exhibit blue-shifted PL, also referred to as an inverse PL shift. The CDs prepared by the template method show a relatively uniform particle size and relatively good water solubility, but the preparation process is complicated, and it is difficult to remove the template completely. The residual template affects the purity and performance of the CDs.

2.2.6. Reflux Method

The reflux method is another method for preparing CDs, but there have been relatively few examples. Liu et al. obtained a clear solution, and it flowed back at 120 °C for 6 h. To better remove the residue, a semi-permeable membrane (MWCO 1000) was used to conducted dialysis on the stock aqueous solution. The aqueous solution obtained by the individual turned golden–yellow, indicating that pure CDs were produced (Figure 4C). Yin et al. used a facile reflux method for the atmospheric pressure preparation of N-GQDs. During the whole experiment, a balloon was placed at the inlet and outlet of the cooler tube, which had two functions: one function was to maintain a relatively closed natural environment and facilitate the inclusion of nitrogen (N) molecules into GQDs and another role was to ensure that the test was conducted under atmospheric pressure. The liquid hue first changed from no color to light yellow and then to orange, indicating that N-GQDs were produced. In contrast to the solid-phase synthesis method, this method uses uniform heating, and the carbon source material is dispersed more uniformly in the solution and shows a higher reactivity. However, be sure to pay attention to the safety factor of the closed reaction system.

3. Basic Photoluminescence Properties

3.1. Optical Absorption

Due to the π-conjugated electrons in its sp² aromatic structure, CDs can effectively capture photons in the short-wavelength region. It shows better electro-optical properties in the UV region (260–320 nm), and the tail of the absorption spectrum can be extended to the visible light region. Generally, the broad peak at ≈230 nm is due to the π–π* transition of C=O bonds in the aromatic structure, whereas the shoulder peak at ≈300 nm or absorption in the visible light region generally originates from the n–π* transition caused by surface C=O bonds or other
functional groups. Functional groups and surface passivation treatment cause differences in structure and composition, which change the absorption characteristics of CDs.

3.2. Photoluminescence Mechanism

Biosensors are widely used in the field of detection because of their high efficiency, sensitivity, and portability. Understanding the detection mechanism of biosensors can help develop new sensors. Zheng et al. synthesized a metal–organic framework (MOF-808) with good peroxidase-like activity under various pH conditions. This framework could catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H₂O₂, accompanied by color changes. The catalytic activity and color change of MOF-808 were related to the concentration of H₂O₂. The measured detection limit of H₂O₂ was 4.5 μM. Ascorbic acid had an inhibitory effect in this process, which allowed to establish a colorimetric sensing method for ascorbic acid. Further research studies found that the catalytic activity of MOF-808 was mainly attributed to the Zr-OH (OH₂) groups, which can be shielded by gluconic acid, thereby reducing the catalytic activity. On the basis of this premise, through the combination of glucose oxidation and TMB oxidation, a colorimetric sensing method for detecting glucose was successfully established (Figure 5A), with a detection limit of 5.7 μM. Fluorescence sensors use the principle that the interaction between the target and the detector causes either fluorescence intensity increase or quenching. He et al. immobilized gold nanoparticles (AuNPs) within iron-based metal–organic gels to obtain a chemiluminescent biosensor with peroxidase-like activity. Fluorescent CDs were obtained in the presence of classic reducing agents, such as acid–base potassium permanganate solution and cerium(IV), and the combination of holes generated in CDs and the radiation source of electronic devices can cause ECL (Figure 6B). This material showed excellent chemiluminescence performance in the presence of H₂O₂. The reason for this chemiluminescence enhancement was that this material can accelerate the generation of OH⁻, O₂⁻, and ¹O₂. Acetylcholine can produce choline in the presence of the catalyst AChE. Choline can produce H₂O₂ with O₂ in the presence of the catalyst choline oxidase (ChOx). The two reactions described earlier were used to control the amount of H₂O₂ produced. Organophosphorus pesticides (Ops) can inhibit the activity of AChE and lead to a reduction in the concentration of H₂O₂, thus affecting the chemiluminescence and enabling the detection of Ops. In addition to the three types of enzyme-simulated biosensors, which we have focused on earlier, there are many categories worth discussing.

3.3. Up-Conversion Glow

Up-conversion luminescent materials can be used in many aspects including biological imaging, biological detection, photodynamic therapy, drug delivery and release, etc. As a fluorescent nanomaterial, it has great potential in the biomedical field. Up-conversion is an anti-Stokes emission process that converts low-energy photons into high-energy photons. According to the up-conversion luminescence mechanism, it can be divided into excited-state absorption (ESA), photon avalanche (PA), energy-transfer up-conversion (ETU), two-photon absorption (TPA), and energy migration-mediated up-conversion (EMU). Next, we will briefly describe the three most common lighting mechanisms: ESA, ETU, and PA (Figure 6A). The principle of ESA is that ions transition from the ground state (G) to an excited

Figure 5. A) Fluorescence spectrum and response area of the sensor system based on CDs. Reproduced with permission. Copyright 2015, Elsevier. B) CDs and related spectrograms. Reproduced with permission. Copyright 2015, American Chemical Society.
state (E1) through ground-state absorption (GSA). E1 absorbs another photon to reach a higher excited state (E2), which then returns to the ground state from the E2 state. Thus, up-conversion luminescence is achieved. Compared with ESA absorption of photons to the excited state (E2), ETU achieves up-conversion luminescence through energy transfer between ions. When irradiated by a suitable excitation source, both adjacent ions reach the E1 state, and then the energy of one ion is transferred to the other ion. This process causes the two ions to reach the E2 state and return to the ground state to complete the up-conversion luminescence. ETU generally requires a higher ion concentration than ESA to achieve luminescence conditions. PA is the combination of ESA and cross relaxation (CR). Through the GSA and ESA processes, the ions transition from the ground state to the excited state. When a metastable state exists, the CR process occurs, causing both ions to occupy the E1 energy level. These two ions then easily occupy the E2 energy level through ESA, resulting in the continuation of the CR process. This process generates sufficient preparation for up-conversion luminescence. Xu et al. used carbonization extraction strategy to prepare N-CDs, which have nitrogen content-dependent polychromatic and diatomic up-conversion characteristics, providing a good idea for optical imaging.

Figure 6. A) Mechanism diagram of ECL and PL in CNCs and ECL response diagram, \( R^+ \), \( R^- \), and \( R^* \) represent negatively charged, positively charged, and excited state CNCs respectively. Reproduced with permission.\(^8\) Copyright 2009, American Chemical Society. B) Dual-wavelength ECL-RET biosensor and related spectrum. Reproduced with permission.\(^{21}\) Copyright 2006, American Chemical Society. C) Electrochemical immunosensing mechanism and correlation diagram. Reproduced with permission.\(^9\) Copyright 2013, Elsevier.
3.4. CD Quenching Mechanism

3.4.1. Static Quenching

Fluorescence quenching refers to a process leading to the whole process of reducing the compressive strength of fluorescent light. It is divided into static quenching and dynamic quenching. During the static quenching process, a complex not-emitting fluorescence is formed between the fluorescent molecule and the quencher, resulting in a decrease in the number of fluorescent molecules and a reduced fluorescence intensity, but no change in fluorescence lifetime is observed. Singh et al. found that Cr(VI) was present and the average lifetime (τ0/τ = 1) was almost unchanged (ignoring the excited-state interaction), therefore confirming the coordination of the excited state between the fluorophore and Cr(VI), that is, static quenching mechanism existed in the system.\[78\]

\[ F_0 - 1 = K[Q] = k_q \tau_0 [Q] \] \hspace{1cm} (1)

where \( F_0 \) and \( F \) are the fluorescence intensity without quencher and with quencher, respectively, \( K \) is the Stern–Volmer quenching parameter, [\( Q \)] is the concentration of the quencher, \( k_q \) is the quenching rate constant, and \( \tau_0 \) is the CDs fluorescence lifetime.\[79\]

The fluorescence mechanism of CDs is relatively complicated. Other than that, FRET quenching mechanism and PET quenching mechanism are both specific mechanisms of dynamic quenching. FRET is considered to be an electric condition, in which the spectrums of the quencher coincides with the spectroscopic analysis method of CDs. In the absence of photons, there is a long-distance dipole-dipole interaction between the quencher and the CDs, and their distance is generally 10–100 Å. Wang et al. proposed a thrombin aptamer biosensor based on FRET. A thombin aptamer (5’-NH2-GGTGGTGTGTTGG-3’) was used to covalently label. The close contact between the kinetic kidney source and the protein kinase causes the fluorescence of UCPs to be quenched. In the presence of thrombin, the aptamer produces a four-strand structure, which weakens the mutual influence, which in turn separates the protein kinase from the kidney source, thereby blocking the entire process of FRET.\[13\] The FRET system between amino-functionalized CDs and AuNPs was used for the detection of melamine (Figure 5B). It had the characteristics of high sensitivity, short analysis time, low cost, and convenient operation. Some CDs combined with −OH and −COOH on the surface, due to the effect of PET, and the electrons excited by light in the CDs were transferred to the empty “d” orbital in the target,\[83\] which suppressed the radiation recombination. As there was no non-radiative electron/hole recombination, the fluorescence of the CDs was quenched. Moreover, single-functional groups produce single-electron transitions. Surface passivation treatment of N-CDs can improve the emission efficiency and help improve the excitation-independent emission properties of the materials.

3.4.2. Dynamic Quenching

Dynamic quenching can be explained by energy transfer or charge transfer mechanisms. The excited state of CDs returns to the ground state through a collision of the quencher with the CDs. Dynamic quenching requires the quencher to be close to the fluorescent molecule when it exists in its excited state and reacts with it, causing the excited state to decay faster, resulting in a shorter fluorescent lifetime but unchanged absorption spectrum. Song et al. reported that the dynamic PL quenching of CD is studied with the trivalent iron system software, and the Stern–Volmer equation is used to analyze PL quenching. Song et al. used ferric ions to exhaustively study the dynamic PL quenching of CDs, and the Stern–Volmer equation was used to analyze the PL quenching.

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\[ F_0 - 1 = K[Q] = k_q \tau_0 [Q] \] \hspace{1cm} (1)

where \( F_0 \) and \( F \) are the fluorescence intensity without quencher and with quencher, respectively, \( K \) is the Stern–Volmer quenching parameter, [\( Q \)] is the concentration of the quencher, \( k_q \) is the quenching rate constant, and \( \tau_0 \) is the CDs fluorescence lifetime.\[79\]

The fluorescence mechanism of CDs is relatively complicated. Other than that, FRET quenching mechanism and PET quenching mechanism are both specific mechanisms of dynamic quenching. FRET is considered to be an electric condition, in which the spectrums of the quencher coincides with the spectroscopic analysis method of CDs. In the absence of photons, there is a long-distance dipole-dipole interaction between the quencher and the CDs, and their distance is generally 10–100 Å. Wang et al. proposed a thrombin aptamer biosensor based on FRET. A thombin aptamer (5’-NH2-GGTGGTGTGTTGG-3’) was used to covalently label. The close contact between the kinetic kidney source and the protein kinase causes the fluorescence of UCPs to be quenched. In the presence of thrombin, the aptamer produces a four-strand structure, which weakens the mutual influence, which in turn separates the protein kinase from the kidney source, thereby blocking the entire process of FRET.\[13\] The FRET system between amino-functionalized CDs and AuNPs was used for the detection of melamine (Figure 5B). It had the characteristics of high sensitivity, short analysis time, low cost, and convenient operation. Some CDs combined with −OH and −COOH on the surface, due to the effect of PET, and the electrons excited by light in the CDs were transferred to the empty “d” orbital in the target,\[83\] which suppressed the radiation recombination. As there was no non-radiative electron/hole recombination, the fluorescence of the CDs was quenched. Moreover, single-functional groups produce single-electron transitions. Surface passivation treatment of N-CDs can improve the emission efficiency and help improve the excitation-independent emission properties of the materials.

3.4.3. IFE Quenching Mechanism

IFE can explain the change in fluorescence intensity with the change in the intensity of excitation light. QY is slightly lower than the fluorescence intensity observed in endlessly dilute solutions. It is likely to cause a decrease in the agitation compressive strength of the entry point or a decrease in the fluorescent compressive strength observed based on digestion and absorption. At this stage, the finite-element method has been applied to the fluorescent cyclodextrin sensing process.\[82\] The CD sensing mechanism is based on IFE, in which the spectra of the absorber and the fluorophore (CDs) excitation/spectral analysis method accurately overlap. Based on the internal filtering effect, fluorophore eliminated the abovementioned complex mechanism and provided a simple and easy countermeasure for the design of the fluorescent sensor. Therefore, an excellent and accurate spectrometer overlap between the absorber Cr (VI) and the nitrogen- and phosphorus-codoped CDs (N- and P-CDs) results in the high efficiency of the IFE process (Figure 5C), resulting in the absorber Cr (VI) to the N- and P-CDs. The excitation light and the emitted light have a shielding effect, which causes the fluorescence to quench.\[78\]

4. Applications of Biosensors Based on CDs

Although research on the applications of CDs is still in the preliminary stage, relatively good progress has been made in some fields, especially in the fields of biomarkers, biosensors, imaging, biochemical analysis, optoelectronic devices, photocatalysis, and drug carriers.\[77,83–85\] A biosensor is an instrument that is sensitive to microbial chemical substances and can convert its concentration value into an electronic signal for testing. It uses immobilized microorganisms and sensitive raw materials as identification elements, including antigens, enzymes, antigens, microbial strains, institutions, somatic cells, nucleotides, and other biologically active chemical substances, and its moderate physics and organic chemical sensors, such as photosensitive tubes, oxygen levels, field-effect tubes. Analysis tools or systems are composed of transistors and signal amplification devices. Biosensors function as receivers and converters. The following section will introduce the application of CDs in biosensing with the hope that more in-depth research will be conducted in the future in this direction.
4.1. Biosensors Based on Fluorescence and Related Techniques

Optical biosensors are biosensors that use antibodies, enzymes, aptamers, and other biological materials as sensing elements and use light signals for detection. Because of their simple operation, good stability, high sensitivity, and fast response, optical biosensors have been used in many detection fields. Optical biosensors mainly include fluorescence biosensors, SERS sensors, and resonance Rayleigh scattering spectroscopy sensors. Fluorescent biosensors mainly use fluorescent materials to form detection probes by electrostatic adsorption, covalent coupling, or avidin–biotin coupling with biological materials and detect an antibiotic content in the sample according to the change in fluorescence intensity. Traditional fluorescent biosensors based on organic dyes easily quench fluorescence, and they exhibit low sensitivity and poor stability. With the development of nanomaterial technology, researchers worldwide have developed many biosensors with long fluorescence life, high fluorescence intensity, adjustable emission spectrum, etc., consisting of new fluorescent nanomaterials, such as QDs, fluorescence microspheres, up-converting fluorescent nanoparticles, etc. The application of new fluorescent nanomaterials has greatly improved the detection sensitivity and stability of fluorescent biosensors. SERS refers to the detection of molecules adsorbed onto the surface of rough noble metal materials, which can enhance the Raman signal of the molecule to be detected. In the Rayleigh scattering experiment, if the wavelength of the incident light is close to the absorption band of the molecule to be detected, the frequency of electron absorbing electromagnetic radiation is the same as its scattering frequency. The electrons rescatter due to resonance and strongly absorb the energy of the scattered light. Compared with a single Rayleigh scattering event, the rescattering–absorption process can increase the intensity by several orders of magnitude and no longer obeys the Rayleigh scattering law, which indicates that the scattering intensity is inversely proportional to the fourth power of the wavelength. This rescattering absorption process is called resonant Rayleigh scattering (RRS). Wang et al. used a thrombin aptamer (5′-NH₂-GTGTGTTGTTGTTG-3′) to covalently label poly(acrylic acid) (PAA)-functionalized up-converting phosphors (UCPs) and bind to the surface of CNPs through π–π stacking. The results showed that the close contact between the energy donor and the acceptor caused fluorescence quenching of the UCPs. Under the best conditions, the fluorescence quenching was 89%. In the presence of thrombin, the aptamer formed a quadruplex-stranded structure, and the π–π interactions weakened, thereby separating the acceptor from the donor which prevented the FRET process. Therefore, the fluorescence of UCPs was restored, which allowed for the concentration-dependent detection of thrombin. In specific applications, the sensor was used to detect the level of thrombin in the body’s blood, and a relatively satisfactory result has been obtained. It is the first time that UCPs and CNPs are used as a kidney-protein kinase pair to build a biosensor based on FRET. The superquenching capabilities of the CNPs and the photophysical properties of the UCPs were combined to provide good analytical performance. Cui et al. successfully synthesized CDs with high QY. GO was quenched by FRET to produce a CD-labeled oligodeoxyribonucleotide. In the presence of Hg⁺, the T–Hg₂⁺–T duplex structure was produced, and β-cyclodextrin was acquired based on the release of CDs-labeled oligodeoxyribonucleotides from GO, and the fluorescence was restored. The fluorescent sensor designed according to this basic principle has the characteristics of easy fabrication, high sensitivity, and high selectivity. It has been successfully applied to the inspection of Hg⁺ in actual samples and used to identify oligodeoxyribonucleosides (Figure 7A). A kind of N-CD synthesized with dopamine as a precursor substance can be used as a promising nuclear staining probe, showing excellent biomolecular simulation performance in cell imaging. Optical sensors are sometimes compared with other sensors, such as N-CDs prepared by microwave-assisted pyrolysis, and used as a dual-sensitive platform for 2,4,6-trinitrotoluene fluorescence detection and electrochemical detection (Figure 7B). The fluorescence sensing platform was based on the strong 2,4,6-trinitrotoluene–amino interaction, which is capable of quenching photoluminescence; the functionalization of amino groups was realized through charge transfer. A glassy carbon electrode modified with cadmium sulfide can reduce 2,4,6-trinitrotoluene, and the minimum distinguishable response concentration decreased to the nanomolar level, implying high specificity and sensitivity. Compared with traditional methods, optical biosensors have characteristics of high sensitivity, faster inspection speed, and simple actual operation. However, characteristics of many types of targets, wide range, low residual amount, and complex sample matrix, are the difficulties limiting the application and promotion of optical biosensors in real-world detection.

4.2. Electrochemical and ECL Biosensors

Biosensing is a method for detecting analytes by combining biological components with physical and chemical detectors. Among the many analysis methods, electrochemical biosensors as an efficient analysis method have many unique advantages, as the actual operation is simple, the sensitivity is high, the selectivity is good, and the cost is low. In the traditional three-electrode electrochemical system, according to the type of biological material attached to the surface of the working electrode, electrochemical biosensors can be categorized into electrochemical DNA sensors, electrochemical enzyme sensors, electrochemical immune sensors, and electrochemical microbial whole cell sensors. The diversification of the CD material structure improves the electronic characteristics and the efficiency of the electrochemical signal transmission at the electrode surface, therefore improving the sensitivity and specificity of the detection method. Some semiconductor nanocrystals, such as low-toxicity carbon nanostructures, can be used as ECL reagents in biochemical applications. Zheng and his colleagues reported a simple and effective approach to prepare water-soluble CNTs with ECL activity using graphite rod scanning potential and observed the ECL behavior during and after preparation. The carbon nanocrystals (CNCs) were completed in an electrochemical cell composed of a graphite rod working electrode, Pt mesh structure counter electrode, Ag/AgCl reference electrode, and pH 7.0 phosphate buffer solution. Figure 8A shows a plan view of the principles of ECL and PL generated in the
carbon nanostructures, which can be used to develop new biosensors and display devices. Gao et al. prepared polyamidoamine dendrimer-terminated CDs using the one-step microwave-assisted thermal cracking of citric acid and polyamidoamine, while simultaneously generating CD surface passivation, and they used the obtained polyamidoamine dendrimer-terminated CDs with colorful amine groups as oxidizing agent and blocking agent. Polyamidoamine dendrimer-terminated CDs/Au nanocrystalline composites were prepared. The resulting nanocomposite material showed good electrical conductivity, the stability and biocompatibility of the electrode surface were designed as a fixed carrier for the sensitive immunosensing of alpha-fetoprotein (Figure 8C). Kavosi et al. published two articles in 2014 and 2015, reporting that dendrimer-encapsulated gold nanoparticles (Au-PAMAM) can be used as hypersensitive α-fetoprotein and prostate-specific antigen biomarkers for prostate cancer ultrasensitive electrochemical immunosensors.

In general, electrochemical biosensors use constant potential, constant current, or impedance to convert biosensing information into monitorable electrical signals. DNA biosensors usually use ssDNA as a probe attached to the electrode surface. When a target DNA molecule with complete complementary pairing is added to the system, the change in the electroactive label or the oxidation of the DNA base caused by hybridization leads to a conversion into electrochemical signals. Gene-related DNA analysis can be achieved by observing the changes in these electrochemical signals. Electrochemical DNA biosensors are widely used due to their powerful analytical capabilities and miniaturization. The graphite-carbon nitride (g-C₃N₄) nanotechnology sheet possesses distinctive photoelectric catalytic properties and has received widespread concern. To date, g-C₃N₄-based nanomaterials have been successfully used to construct new electrochemical biosensors to facilitate a variety of biological detections, including DNA sensing, immunoassays, and small-molecule detection. Feng et al. used the RET between g-C₃N₄ nanosheets and Ru(bpy)₃²⁺, designed a ratio-dependent ECL analysis method, and achieved high-sensitivity detection of miRNA. As shown in Figure 8B, when AuNPs were used to functionalize the g-C₃N₄ nanosheets, the resulting Au–g-C₃N₄ complex showed a stable ECL emission peak at 460 nm. This ECL emission coincides with the digestion absorption peak of Ru(bpy)₃²⁺, which stimulated the emission of Ru(bpy)₃²⁺ at 620 nm. The strength of the ECL data signal at 460 nm was...
reduced and the signal at 620 nm was increased, forming a dual-wavelength ratio-dependent ECL–RET sensing system, in which quantitative detection of miRNA can be achieved by measuring the ratio of ECL460 nm/ECL620 nm. [21] Electrochemistry can also be used in combination with a variety of technologies, such as molecularly imprinted polymer-modified glassy carbon electrode for the determination of isoacetone in water. [90] In the three-electrode system, the functionality of the electrochemical sensor is mainly based on the electron transfer between the active material on the working electrode and the target analyte. When the material is used as the electrode material, its electrochemical activity, specific surface area, and conductivity are the main factors determining the performance of the electrochemical sensor. Compared with the corresponding bulk materials, the smaller size, large surface area, and high signal-to-noise ratio of nanomaterials improve the properties of the materials to function as electrochemical sensor materials. Ultrathin 2D nanomaterials show good conductivity, excellent electrochemical properties, and ultrahigh specific surface area, so they are widely used as electrode materials and applied in electrochemical sensing research.

4.3. PEC Biosensors

The PEC biosensor is an effective tool to detect biological events related to cells. Biological phenomena and signal converters are used to convert biological phenomena into photocurrent signals. The changes in these photocurrent signals are used to achieve qualitative and quantitative analysis of the target. Compared with fluorescence, electrochemical, and ECL technologies, PEC detection technology has gained substantial application value due to its independent excitation light source and signal acquisition system, which shows a low background signal and high detection sensitivity. The main components of PEC biosensors are divided into photovoltaic active materials and biological recognition systems. The function of the photovoltaic active material is the generation of photocurrent under the excitation of light of a certain wavelength. Commonly used photovoltaic active materials include inorganic semiconductor nanomaterials, organic small molecules and polymers, and noble metal nanoparticles. Among these materials, CDs and their composite materials have attracted the greatest attention. Combined with the advantages of PEC detection technology, the use of different types of
biorecognition elements to construct PEC biosensors for the detection of DNA, biological enzymes and cells, and other targets has become a research topic of great concern in the field of PEC analysis. The current research on PEC biosensors mainly focuses on expanding the scope of optoelectronic materials, determining effective sensing strategies, improving the detection limits, and developing sensors for clinical applications. Liu et al. developed a PEC lactate dehydrogenase biosensor based on MWCNT–TiO$_2$ nanoparticles composite platform. The holes produced by the illuminated composite can regenerate NAD$^+$ and complete the catalytic cycle of the enzyme efficiently and quickly. The MWCNTs in the composite materials promoted electron transport away from the TiO$_2$ nanoparticles and therefore prevented their recombination with the holes; the composite material was not only an NAD$^+$ regeneration tool for PEC dehydrogenase biosensors but also a biocompatible matrix. Liu et al. developed low-toxicity CDs combined with synthesized AuNPs. Based on the excellent PEC activity of the CD–cysteamine-terminated AuNP conjugates, a label-free PEC cell sensing strategy was developed (Figure 9A). The localized SPR of the AuNPs excited the CDs to generate more electron–hole pairs. Based on the excellent photovoltaic performance of composite materials, the separation of electron–hole pairs and interface-induced thermal electron transfer were used for sensitive detection of HeLa cells. After that, in the presence of Bi(Ac)$_3$ and oleylamine, the same research group successfully synthesized bismuth@N-,O-codoped carbon core–shell nanohybrids through low-temperature liquid carbonation of FA, and a PEC biosensor based on this complex was used for ultrasensitive detection of telomerase in HeLa cells (Figure 9B). The PEC sensor combines the advantages of optical and electrochemical sensing, so it has major application prospects in the field of analysis. However, there are still many challenges limiting the application of CDs in PEC biosensors. For example, we have to expand the kinds of photoelectric materials, develop photoelectric electrode materials with stable photoelectric current performance and strong photoelectric response to meet the research needs, and develop biological elements with strong exclusive recognition ability by simplifying and optimizing the construction strategy of the sensing platform and improving the sensitivity and stability of detection.

4.4. QCM-Based Biosensors

A QCM is an instrument that uses quartz crystals as an energy transducer and uses their piezoelectric effect to transform the mass signal of the substance into a frequency signal, thus allowing for detection of mass and concentration. The measurement accuracy can be up to the order of nanograms. The development...
of QCM instruments was very rapid, from the early conventional QCM to flow QCM, dissipative QCM, array QCM, and electrochemical QCM combined with electrochemical detection. Curie discovered in 1880 that internal polarization occurs when a quartz crystal is deformed by an external force in a certain direction; at the same time, positive and negative charges are generated on its two opposing surfaces. If the applied electric field is an alternating electric field, the crystal produces mechanical oscillations. When the frequency of oscillation coincides with the natural frequency of the crystal, resonance occurs. At this time, the oscillation is most stable, and its oscillation frequency is the natural frequency of the crystal. These led to the initial assumptions of QCMs. An idealized model of QCMs was described by Günter Sauerbrey, who proposed a useful empirical method to describe the change in the resonance frequency of quartz crystals as a function of the mass of the substance added to the surface. However, in the past decade, an increasing number of QCM sensors with anti-Sauerbrey behavior was developed due to the interaction between the target analyte and the sensitive layer, and instead of a decrease in the QCM response, as predicted by the Sauerbrey equation, a positive resonance shift was observed as the mass increased. Lien et al. reported on the electrochemical detection of the DNA of genetically modified organisms based on polypyrrole doped with MWCNTs. The DNA hybridization was studied by QCM and electrochemical impedance spectroscopy (Figure 10A). QCM technology can detect targets as low as 4 pM. The system is not only suitable for DNA but also suitable for the construction of protein biosensors. Wang et al. developed a device for detecting and monitoring the response of living cells to engineered nanomaterials in real time. The device is a living-cell QCM biosensor, which uses macrophages adhering to quartz crystals. The generated response of the macrophages to the treatment was continuously monitored though the changes in the crystal oscillation frequency. The researchers reported the ability of this QCM biosensor to distinguish between benign and toxic exposures and revealed unique cell response kinetic information at different doses of single-walled CDs. QCM has the advantages of simple structure, low cost, high resolution, high sensitivity, good specificity, and real-time online monitoring. It is widely used in various fields, such as physics, biology, chemistry, and medicine.

Figure 10. A) Colorimetric biosensor absorption spectrum and linear calibration plot. Reproduced with permission. Copyright 2018, American Chemical Society. B) CDs spectrogram and fluorescence spectrum of the CDs-AuNP system. Reproduced with permission. Copyright 2014, Elsevier. C) Mechanism of IFE and static quenching sensitivity to Cr(VI) and ascorbic acid and N,P-CD fluorescence spectra. Reproduced with permission. Copyright 2018, The Royal Society of Chemistry. D) Electron transfer mechanism and linear range when CDs detect Hg$^{2+}$. Reproduced with permission. Copyright 2016, The Royal Society of Chemistry.
4.5. SPR-Based Biosensors

Generally, when the size of the AuNP is much lower than the light wavelength of the incident angle, it will experience a relatively uniform electrostatic field, causing the collective oscillation of its conducting electrons. This oscillation creates an energy-dependent resonance called SPR. SPR sensors combine biological recognition mechanisms with optoelectronic devices to convert biological signals into optoelectronic signals, which is very suitable for dynamic real-time research on biomolecular interactions. During the early stages, the red shift of the SPR band to 605 nm was reported to be affected by the shape, size, and carbon coating of the synthesized C/Cu. When metal nanoparticles are excited by electromagnetic radiation, the collective oscillation of their conducting electrons takes the form of surface SPR, and surface plasmons (SPs) are distributed on the surface of the particles.\[12\] At present, SPR sensors have become the mainstream technology for real-time observation of biomolecules and an important detection tool for the interaction of biomolecules. In many of the SPR biomass-sensing applications, wavelength-indicating SPR sensors are used, especially a direct spectrometer scanning scheme to obtain spectra for subsequent demodulation. This scheme has a simple optical path and good stability; the sensitivity does not depend on the characteristics of the sample; it is suitable for long-term large-dynamic-range experiments, and the sensitivity of typical work is high. The intensity-indicating scheme obtains a faster demodulation speed and may obtain higher sensitivity. However, for light intensity measurement at a fixed wavelength, the large-scale movement of the sample spectrum reduces the sensitivity, so it is only suitable for areas with a small dynamic range, such as presence detection. To improve the accuracy of the angle indicating the SPR sensor, the coupling optical path is often more complicated and requires more subsequent processing, and the measurement range is also affected by the optical path design. The SPR biosensor can be used to detect L. monocytogenes by phage display antibodies, imaging, and other aspects. Park et al. used SPR imaging technology to study the adsorption kinetics of single-stranded DNA-dispersed single-walled CDs on an immobilized collagen layer.\[93\] Zhang et al. reported a method for the layer-by-layer self-assembly of an inhibition enzyme internalized in a MWCNT-containing acetylcholinesterase (AChE) biosensor.\[14\] Lee et al. described a method for amplifying biosensor signals in SPR-based immunoassays using antibody–CNT conjugates. Taking human hemopoietin and human granulocyte macrophage colony-stimulating factors as the solid model system software, SPR biosensors were used for sandwich-type immunodetection (Figure 10B). To amplify the SPR signal, CNTs were combined with a polyclonal antibody and then reacted with the antibody to obtain the target protein,\[94\] and this amplification strategy not only increased the dynamic range of immune markers but also improved the detection sensitivity. Jiang et al. reported a monodispersed bismuth nanoparticle-modified g-C₃N₄ photocatalyst and a highly efficient method for the removal of divalent nitrogen in continuous cyclones under direct visible light in ppb.\[95\] This result may be the reason for the increased removal efficiency at the origin of polydispersed NPs on g-C₃N₄ due to SPR and the e⁻/h⁺ increase induced by SPR and polydispersed g-C₃N₄ heterojunction. Essner et al. reported a CD-based sensor with good catalytic effect with the reaction of 4-nitrophenol.\[96\] During the experiment, at a fixed concentration of HAuCl₄ of 0.3 mM, along with the increased CD concentration, the compressive strength of SPR increased and a blue shift occurred (indicating that AuNPs were small) (Figure 10C). When the CDs concentration reached 0.3 mg mL⁻¹, the compressive strength of SPR remained relatively stable, and the wavelength shift was negligible. When the concentration of CDs was constant, increasing the amount of HAuCl₄ increased the intensity of the SPR and a pronounced red shift was observed (indicating that AuNPs are larger), indicating the potential tunability of the resulting AuNPs. These results confirmed that by simply changing the ratio of CDs: Au, it is possible to easily fine tune the SPR. The red shift and peak width in SPR spectroscopy are mainly attributed to the increase in particle size and the increase in polydispersity. Theoretical research and experimental work implied that the SPR is affected by many factors, including the size, concentration, and shape of the metal particles and the dielectric properties of surrounding material. In some studies, SPR sensors have also been used in conjunction with other sensors, such as near-infrared sensors, and have achieved relatively good results.\[96\]

SPR technology has the characteristics of convenient and fast detection process, high sensitivity, not needing to mark the samples, and in most cases, not needing to process the samples, and it can be conducted in turbid or even opaque samples.

4.6. Other Biosensors

In recent years, thermal sensors based on carbon electric materials, piezoelectrics, field-effect tubes, and other biosensors have also been widely used. Piezoelectric biosensors are new biosensors, which use piezoelectric materials as transducers. In recent years, piezoelectric bioreactors have been characterized by their sensitive response, high specificity, simplicity, and speed; there was no need for labeling of the samples as well as easy automation and integration of methods occurred. Field-effect tube biosensors have been successfully used for the detection of various biomolecules, for instance, proteins, nucleic acids, and sugars have the advantages of ultrahigh sensitivity, high specificity, label-free nature, and instant response. However, compared with other nanomaterials, the application of CDs for these types of sensors is still relatively small; applying CDs to this area can be a future development direction worth exploring.

5. Conclusion and Outlook

This article summarized the synthesis methods of CDs and how to make them in the field of sensing. Although many methods for synthesizing CDs have been proposed, there are still some limitations regarding the synthesis of CDs. For example, synthesized CDs are faced with the problem of low QY. The structure and size of controlled synthesis and large-scale synthesis are still difficult to achieve. Although surface passivation of CDs with appropriate functional groups can help improve the QY, surface passivation is a complex and time-consuming process. Nitrogen has an atomic size similar to C atoms and five valence electrons.
and can be combined with C atoms. Nitrogen doping can also effectively adjust the surface chemical activity, electronic properties, and optical properties of CDs. Therefore, it is worth developing the simple and reasonable introduction of nitrogen atoms and other heteroatoms to synthesize doped CDs. The research interest in CDs has exceeded beyond traditional fields of biosensors, such as bioimaging and biomimetic. Individual CDs are still limited in terms of their large-scale application. CDs can be combined with other raw materials according to different needs to obtain better development prospects.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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