Biomass-derived carbon dots as emerging visual platforms for fluorescent sensing

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Abstract:

Biomass-derived carbon dots (CDs) are non-toxic and fluorescently stable, making them suitable for extensive application in fluorescence sensing. The use of cheap and renewable materials not only improves the utilization rate of waste resources, but it is also drawing increasing attention to and interest in the production of biomass-derived CDs. Visual fluorescence detection based on CDs is the focus of current research. This method offers high sensitivity and accuracy and can be used for rapid and accurate determination under complex

conditions. This paper describes the biomass precursors of CDs, including plants, animals and microorganisms. The factors affecting the use of CDs as fluorescent probes are also discussed, and a brief overview of enhancements made to the preparation process of CDs is provided. In addition, the application prospects and challenges related to biomass-derived CDs are demonstrated.

Keywords: Biomass conversion, carbon dots, visual platforms, fluorescent probe, fluorescent mechanisms

1. Introduction

The fluorescence sensor is a compound that contains a binding site, a fluorophore, and a communication mechanism between two sites (Brzechwa-Chodzyńska et al., 2021). The mechanism of detection is a probe that detects the object based on changes in optical properties. These sensors boast high sensitivity and accuracy, and they have broad application prospects in food analysis (Liu et al., 2019b; Luo et al., 2020; Pan et al., 2020), metal ions detection (Mehta et al., 2021; Yang et al., 2020) and other fields (Shin et al., 2021). Furthermore, research in the area of fluorescence sensor development targets cheap and simple structure (Zhang et al., 2023a). Traditional detection methods rely primarily on fluorescence spectrometers. However, this approach requires equipment and professional personnel, and the cost is higher. However, fluorescent sensing can be designed as an emerging visualization platform. The visualization method is accomplished with the help of simple, inexpensive instruments, such as the use of test strips or smartphone apps to facilitate testing (Castro et al., 2021). The advantage is that the reagent consumption is low, and such devices can be easy to carry. Yu et al., for example, used a portable fluorescent device for field detection. In their approach, the fluorescence signal is captured by a smartphone camera and converted into an image, and quantitative analysis is completed by the red, green, and blue (RGB) value of the image (Liang et al., 2022). Fluorescent sensors have been widely used, but there is

still room for further development in biomedical (Chen et al., 2021; Duo et al., 2023) or microenvironment monitoring (Shin et al., 2021; Yin et al., 2021). To improve the detection efficiency of fluorescence sensors, the method of coupling high-performance materials (De Acha et al., 2019) or absorptive fluorescence modulation of solid surface can be employed (Yang et al., 2014).

Carbon dots (CDs) are carbon-based zero-dimensional materials with good photostability and biocompatibility (Meng et al., 2019). CDs have important applications in fluorescence sensing, various optical devices (Castelletto and Boretti, 2023; Li et al., 2023c), electrode materials (Niyitanga et al., 2023), and room temperature phosphorescent materials (Hu et al., 2023; Li et al., 2023b). CDs are a kind of carbon hybrid nanomaterial, mainly composed of carbon quantum dots (CQDs) (Rasal et al., 2021), graphene quantum dots (GQDs) (Biswas et al., 2021), and carbonized polymer dots (CPDs) (Kang et al., 2022). GQDs consist of one or more layers of graphene whose edges are connected by chemical groups (Ai et al., 2021). CPDs are crosslinked polymer or polymer chains based on monomers or linear polymers (Ai et al., 2021). CQDs have a graphite lattice structure with distinct lattice fringes (Ai et al., 2021; Innocenzi and Stagi, 2023). GQD precursors are essentially graphitized, such as graphite, carbon nanotubes, etc., whereas CQDs are composed of carbon-rich precursors, and there are more kinds of precursors for CQDs (Deka et al., 2022). A variety of raw materials are used in the preparation of carbon materials, including graphite, small molecules, polymers, and natural materials (Xia et al., 2019). Among these, biomass materials are rich in carbon and a variety of other

elements (Abbas et al., 2018; Wang et al., 2021b), and preparing carbon materials as precursors can reduce the use of chemical reagents. Biomass materials include plants, animals, microorganisms, and so on. Plants are popular precursors for the preparation of carbon materials and are rich in cellulose, hemicellulose, and lignin (Wang et al., 2021b). Animal bone and animal protein can also be used as precursors for carbon materials, and their derived materials contain amino acids, proteins and trace elements (Fan et al., 2022). The processing methods of biomass conversion into carbon materials include hydrothermal carbonization, pyrolysis, microwave radiation, catalytic conversion, etc. (Malode et al., 2023a; Wang et al., 2021b).

The production of carbon materials from biomass materials not only improves the utilization rate of waste materials, but it also reduces the cost of carbon material preparation. For the disposal of waste, the incineration or random placement of agricultural waste and residues can cause environmental pollution problems (Abbas et al., 2018). By using renewable, cheap biomass as a carbon source, we can both maximize the use of energy and create new avenues for energy utilization. Biomass material as a precursor makes green synthesis simpler, and the material comes from a wide range of sources, doped by a variety of atoms (Fan et al., 2022). Biomass derived CDs offer low cost, low toxicity and environmental friendliness, and they can be used for large-scale preparation and biomedicine. Compared with non-biomass CDs, the heteroatoms in biomass can achieve self-doping, which gives the CDs excellent properties (Fang et al., 2024). In addition, compared to other fluorescent probes, such as organic dyes (rhodamine, cubic acid), semiconductor quantum dots,

and precious metal nanoclusters, biomass derived CDs do not contain heavy metal elements and have good biocompatibility. Moreover, these CDs have the advantages of low cost, good light stability, strong photobleaching resistance, and good water solubility (Li et al., 2021d; Liu et al., 2024; Mathew and Mathew, 2023b; Zhang et al., 2022b).

Previous research efforts have discussed the synthetic and fluorescent properties of CDs, as well as their applications in food analysis and detection of metal ions (Li et al., 2023a; Yoo et al., 2019). Biomass CDs are also mainly focused on synthetic pathways, fluorescence mechanisms, and application (Jing et al., 2023; Liu et al., 2019a). However, the significance and innovation of this research are focusing on the influencing factors and improvement of fluorescence sensing. The detection mechanisms are summarized, and the improvement of biomass-derived CD preparation is provided. The effects of pH, temperature, reaction time, and reactant ratio on the application of CDs are studied. Moreover, the optimization of reaction conditions, the use of sustainable carbon sources, and element doping are briefly discussed. In addition, the precursors of biomass-derived CDs are briefly described and summarized. Finally, the prospects and challenges related to biomass-derived CDs are presented (**Fig. 1**).

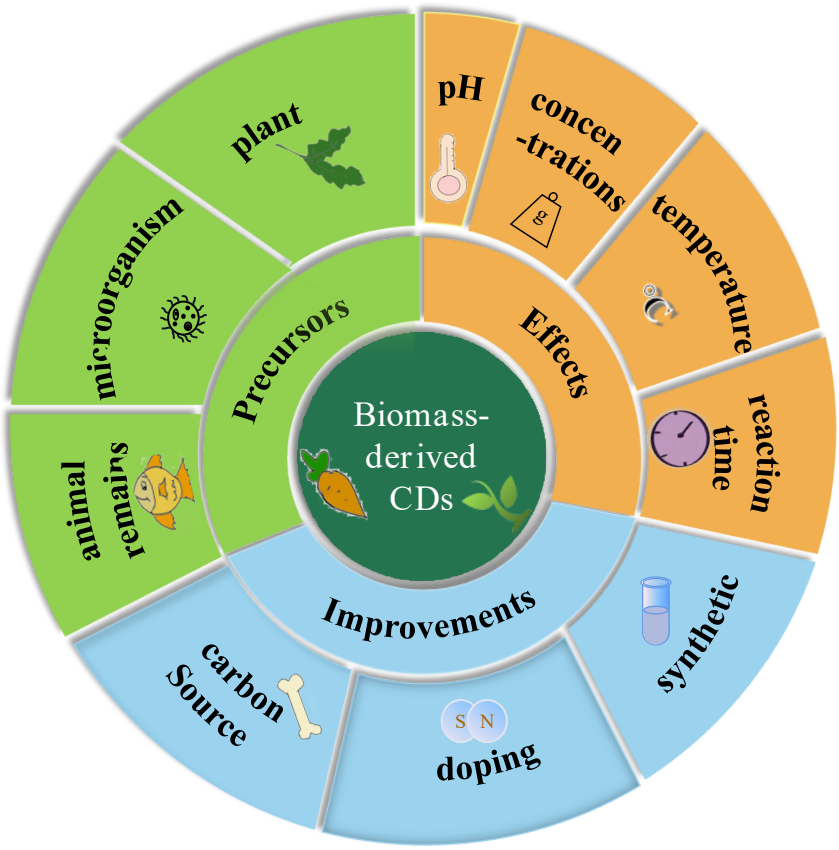

Figure 1. Precursors, effects, and improvements of biomass-derived CDs.

2. Mechanisms of fluorescent sensors

2.1 Aggregation-induced emission mechanism

Fluorescence sensing has the advantages of good sensitivity, fast response speed, and low cost. When the concentration of the analyte changes the fluorescence properties, the system can be considered for sensing (Deka et al., 2022). Various mechanisms have been proposed to explain fluorescence sensing (**Table 1**). Aggregation-induced emission (AIE) is a general strategy designed for sensing

systems. The fluorophores induced by fluorescence aggregation have the advantages of high emission efficiency, strong light stability, and large Stokes shifts. The study mechanism of AIE includes restriction of intramolecular motion, excimer formation, polymerization, etc. Of these, the restriction of intramolecular motion may specifically refer to constraints on intramolecular rotation and vibration (Gao and Tang, 2017). Many luminous materials display AIE properties and can be pure hydrocarbons, organometallic molecules, polymers, and so on (He et al., 2018). For CDs, the chemical stability as well as the tunable and up-photoluminescence behavior of CDs offer great potential in the application of fluorescence sensors (Liang et al., 2022; Salimi Shahraki et al., 2022). AIE phenomena were shown to occur in CDs prepared by Yang et al., in which Polyethylenimine (PEI) and citric acid were used as raw materials for the preparation of CDs. After adenosine triphosphate (ATP) was added, the fluorescence intensity and quantum yield of CDs were enhanced. The AIE phenomenon was determined to be a result of the coordination and electrostatic interaction between ATP and prepared CDs. Based on the AIE phenomenon, a simple ATP detection platform was established (Huang et al., 2022b). In addition, the literatures on materials detection based on the mechanism of AIE is extensive (Li et al., 2021b; Zhang et al., 2023b). Substances with AIE characteristics have significant application prospects in sensing and bioimaging (Niu et al., 2020), as well as in medical (Gao and Tang, 2020) and other fields. However, when they are applied in complex environments, more stringent requirements are imposed with respect to specificity and sensitivity (Wang et al., 2023b). Achieve these two requirements is a

156 challenge in the process of material exploration.

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158 **Table 1.** Applications of CDs under different mechanism types.

Mechanisms	CDs	Detector	Read Out	Linear range	LOD	Ref.
AIE	Nitrogen-doped CDs	ATP	Turn-on	1–2000 μM	0.8 μM	(Huang et al., 2022b)
	Red fluorescent CDs	Tetracycline antibiotics	Turn-on	3−35 μM (TC)	12 nM (TC)	(Li et al., 2021b)
	Tea saponin-CDs	Human serum albumin	Turn-on	0–180 μM	140 nM	(Zhang et al., 2023b)
Static quenching	Nitrogen-doped CDs	Curcumin	Turn-off	0.1–20 μM	29.8 nM	(Tang et al., 2021a)
	CDs	Sunset yellow	Turn-off	0.3-56 μmol L^{-1}	74 nmol L^{-1}	(Yang et al., 2018)

	O-CDs	Morin; ClO^-	Turn-off	5–125 μM (morin); 2.5-90 μM (ClO^-)	0.8 μM; 0.5 μM	(Meng et al., 2022)
	Nitrogen-doped grape peels CDs	Baicalin	Turn-off	0.1-20 μM	43.8 nM	(Tang et al., 2022)
Dynamic quenching	N,S-BCDs	Cr (VI)	Turn-off	0-400 μM	0.2 μM	(Wang et al., 2022 a)
	N-CDs (citric acid+benzoylurea)	Fe(III)	Turn-off	30-100 μM	1.1 μM	(Shan et al., 2021)
	N-CDs	p-nitrophenol	Turn-off	0.5-70 μM	0.2 μM	(Liao et al., 2021 a)
FRET	CD	Oxytetracycline	Turn-on	0-40 μM	0.4 μM	(Fu et al., 2021)
	CDs	Dithizone	Turn-off	—	—	(Liu et al., 2020)
IFE	CDs-MnO_2 nanosheets	Dopamine	Turn-on (elimination of IFE)	1-30 μM	100 nM	(Tan et al., 2020 b)

N-CDs	MnO_4^-	Turn-on	0.2-9.0 μM	0.1 μM	(Chen et al., 2022a)
CNF-TCDs3	Cr(VI)	Turn-off	0-15 mg/L	0.2 mg/L	(Hu et al., 2022)

2.2 Static quenching mechanism

Various fluorescence changes (e.g., intensity, wavelength, lifetime) of CDs are relevant to their potential as sensors, and these changes are also related to the fluorescence sensing mechanism (Sun and Lei, 2017). Static quenching is the interaction of probe and quencher to form a non-fluorescent ground state complex (Liu et al., 2019a). Fluorescence quenching occurs when CDs are used as probes to detect substances, and the possibility of static quenching should be considered. The nitrogen-doped fluorescent CDs (NCDs) prepared by Yu et al. can be used to detect curcumin, and the mechanism of detection involves static quenching and internal filtration effects (Tang et al., 2021a). The fluorescence lifetime of NCDs did not change significantly after curcumin was added, and the quenching type was determined by using the standard Stern-Volmer equation. In addition, Hu et al. prepared CDs via a hydrothermal process, which can be used to detect sunset yellow (Yang et al., 2018). The quenching mechanism is also studied with the help of the Stern-Volmer equation $F_0/F = 1 + K_q\tau_0[Q] = 1+K_{sv}[Q]$. Here, K_q is the quenching

constant, and K_{sv} is the Stern-Volmer quenching constant. F and F_0 are the fluorescence intensity of CDs before and after adding sunset yellow, τ_0 is the fluorescence lifetime before adding quencher, and [Q] is the concentration of adding quencher. Static quenching can be distinguished according to the change of the quenching constant at different temperatures. In this system, the quenching constant decreases with the increase in temperature, which confirms that the mechanism is static quenching. The fluorescence lifetime did not change significantly before and after adding sunset yellow, which further proves that the quenching mechanism is static quenching.

2.3 Other mechanisms

According to the fluorescence quenching phenomenon, the mechanism of the analyte detection includes dynamic quenching (Zu et al., 2017), Forster resonance energy transfer (FRET), inner filter effect (IFE), and so on (**Fig. 2**). FRET is a non-radiative energy transfer process between donor-acceptor pairs (Wu et al., 2020). IFE refers to the effect in which the observed fluorescence intensity in a system is proportional to the intensity of the excited light, and the quantum yield is slightly lower than that of infinite dilution (Chen et al., 2018; Li and Zhang, 2021; Li et al., 2021d). IFE production may be caused by concentration effects or by the capture of the excited light by the absorbent and the emitted light of the fluorophores, resulting in reduced fluorescence (Li and Zhang, 2021). The practical application of CDs cannot be separated from the support of fluorescence sensing theory. The fluorescence

mechanism of CDs can be explained by molecular state, surface state, size, and other theories. As the degree of CDs carbonization increases, core-shell structure is gradually formed. The fluorescence mechanism of CDs in the formation of core-shell structures is the most widely studied (Wang and Lu, 2022). The application of CDs is based on changes in fluorescence caused by the interaction of the analyte with CDs (Zu et al., 2017). In addition, CDs as a probe can also be used to detect molecules and anions, as shown in **Table 2**. Moreover, carbon material-based fluorescence sensors have been shown to effectively detect targets in organisms and the environment, using bioimaging technology or combined with smartphones to achieve real-time monitoring of targets (Li et al., 2023a). FRET, IFE, and static quenching mechanisms have been widely used in detection using CDs as fluorescent probes (Wang and Lu, 2022). AIE uses a turn-on model to analyze species, while AIE luminogens has low background noise and good light stability (Wang et al., 2023a).

Table 2. Detecting anions and molecules by fluorescence detection mechanism.

	Detector	CDs	Mechanism	Ref.
Anion	ClO^-	N-CQDs	Dynamic quenching	(Jiang et al., 2020)
	MnO_4^-	N-CDs	Inner filter effect	(Chen et al.,

				2022b)
	ClO^-	N,Cl-CDs	Dynamic quenching; photoinduced electron transfer	(Liu et al., 2021b)
	S^{2-}	N, S-CDs	High affinity between S^{2-} and Sn^{2+}	(Liao et al., 2023)
	PO_4^{3-}	GQDs	Strong affinity between PO_4^{3-} and Ce^{4+}/Fe^{3+}	(Wang et al., 2022b)
Molecule	Adenosine triphosphate	Nitrogen-doped CDs	Aggregation-induced emission	(Huang et al., 2022a)
	Curcumin	Nitrogen-doped CDs	Static quenching	(Tang et al., 2021b)
	Tetracycline antibiotics	Red fluorescent CDs	Aggregation-induced emission	(Li et al., 2021c)

p-nitrophenol	N-CDs	Forster resonance energy transfer	(Liao et al., 2021b)
Dopamine	CDs-MnO_2 nanosheets	Inner filter effect	(Tan et al., 2020a)

The mechanism by which CDs are applied to fluorescence sensing is described above. As fluorescent probes, CDs can be used to detect molecules (thiols, antibiotics, nitroaromatics, proteins), ions (Fe^{3+}, Hg^{2+}, Cu^{2+}, $S_2O_3^{2-}$, ClO^-, PO_4^{3-}), pH, temperature and so on. Biomass CDs have good response to a variety of substances and can be used for the detection and analysis of actual samples (Liu et al., 2019a; Malode et al., 2023a). In industrial engineering, biomedical monitoring and environmental control, temperature and pH measurements are the basis for ensuring efficient and reliable operation of the system in question. As substances that are sensitive to temperature and pH, CDs have great research value (Yadav et al., 2023; Zhang et al., 2019).

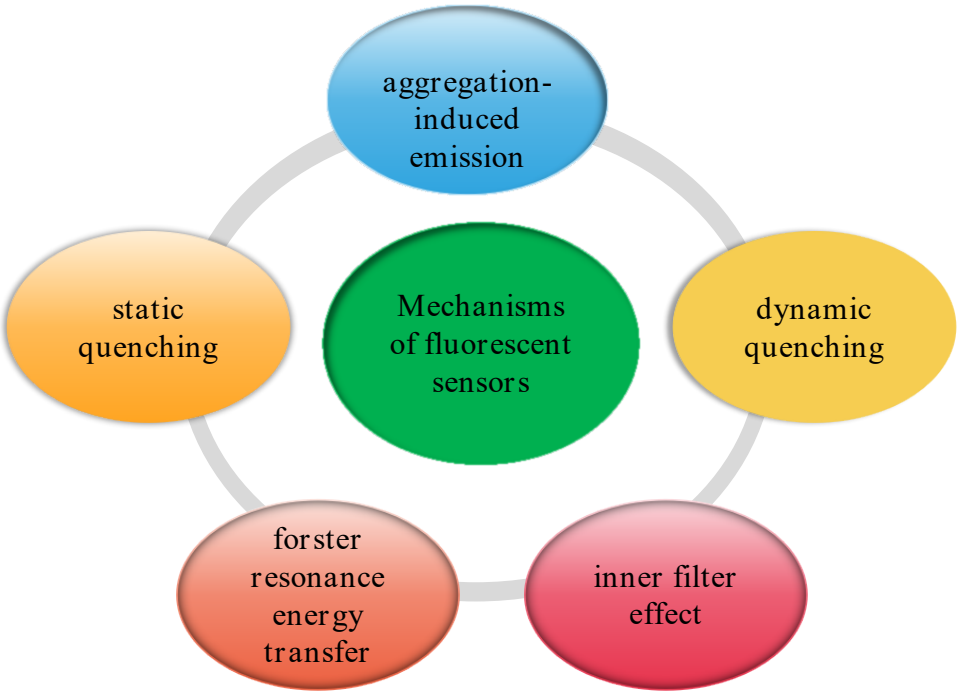

Figure 2. Mechanisms of fluorescent sensors

3. Precursors in biomass-derived carbon dots

3.1 Structure and preparation of carbon dots

The analysis of CDs structure is challenging because CDs vary in size and optical properties, which complicates the interpretation of structural data. In addition, determining whether the captured signal comes from the surface or the core is a problem (Mintz et al., 2021). The crystal properties of CDs can be further studied by high-resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD), and other techniques (Liu et al., 2019a). CDs synthesis involves breaking

apart chunks of material or assembling atoms or molecules to form nano-sized particles (Kumar et al., 2022). CDs can be prepared by hydrothermal synthesis (Perumal et al., 2021), microwave-assisted synthesis (Ng et al., 2021), chemical oxidation (Meng et al., 2019), ultrasonic synthesis, among other techniques. The hydrothermal method can use a variety of biomass materials as a carbon source, which has the advantages of environmental protection and low cost (Perumal et al., 2021). Microwave-assisted synthesis can shorten the hydrothermal time, but the quantum yield cannot be improved significantly (Ng et al., 2021). Chemical oxidation can use strong oxidizing acids to carbonize organic matter; however, the harmful effects of strong oxidizing acids on the environment limit the application of this method (Jing et al., 2023). The method of obtaining raw materials from nature to prepare CDs is green and pollution-free, and the reuse of waste materials (e.g., peel, bone) also addresses the problem of household waste disposal.

Common synthesis methods of biomass derived CDs include hydrothermal method, electrochemical oxidation, chemical oxidation, microwave, and pyrolysis. To improve the fabrication of biomass-derived CDs, ball mill can be used to treat the material in the raw material pretreatment process, and the consistent particle size can be tuned by controlling the parameters. To make biomass derived CDs with special functions, carbon sources with special structures can be selected. It is also possible to change the morphology or properties of CDs by changing reaction solvents, or to change the optical and electronic properties via element doping. For the reaction solvent, its dewatering ability, polarity, and proton properties all affect the properties

of CDs (Gan et al., 2023; He et al., 2023; Malode et al., 2023b; Mathew and Mathew, 2023a; Miao et al., 2020; Rasheed, 2023).

3.2 Plant-based biomass

In the process of CDs synthesis, biomass materials are selected as carbon precursors, which are advantageous because they are non-toxic and low cost (Cao et al., 2022b; Deng et al., 2021; Feng et al., 2021). Both plant and animal remains can be used as materials to synthesize CDs (**Table 3**). Likewise, plant residues have great potential in the synthesis of CDs (Arumugham et al., 2020; Gupta et al., 2020; Li et al., 2018; Shen et al., 2017). Shang et al. prepared CDs by a hydrothermal method using Toona sinensis leaves and ethylenediamine (EDA) as raw materials (Bei et al., 2023). To improve the sensitivity, a ratio fluorescence probe (CDs@5-FAM) was constructed using CDs and 5-carboxyfluorescein (5-FAM). In this method, as the concentration of urea increases, the fluorescence changes from blue to green under UV light (**Fig. 3A**). Sawant et al. for the first-time prepared biomass-derived CDs using banana petioles as precursors. The CDs can be used as a sensing probe for Fe^{3+} detection with a detection limit of 0.21 nM. The detection mechanism is photoinduced electron transfer to form a non-radiative charge transfer complex. In addition, solution and paper-based analysis methods were designed based on smart phones. The relationship between the average color intensity and iron concentration was obtained by analyzing the image with software (Korram et al., 2023). (**Fig. 3B**).

Furthermore, Fu et al. obtained high fluorescence CP-CDs from rice fried

Codonopsis pilosula (CP) by ultrasonic-assisted solvent extraction at room temperature (Qiu et al., 2020). CP-CD production process was simple, convenient, and green. CP-CDs showed high light stability and could be used for the detection of Cr (VI) in environmental sewage samples. CP is a common Chinese medicine. Therefore, making CP-CDs with CP reduces the cost of synthesis and expands the application value of traditional Chinese medicine resources (**Fig. 3C**).

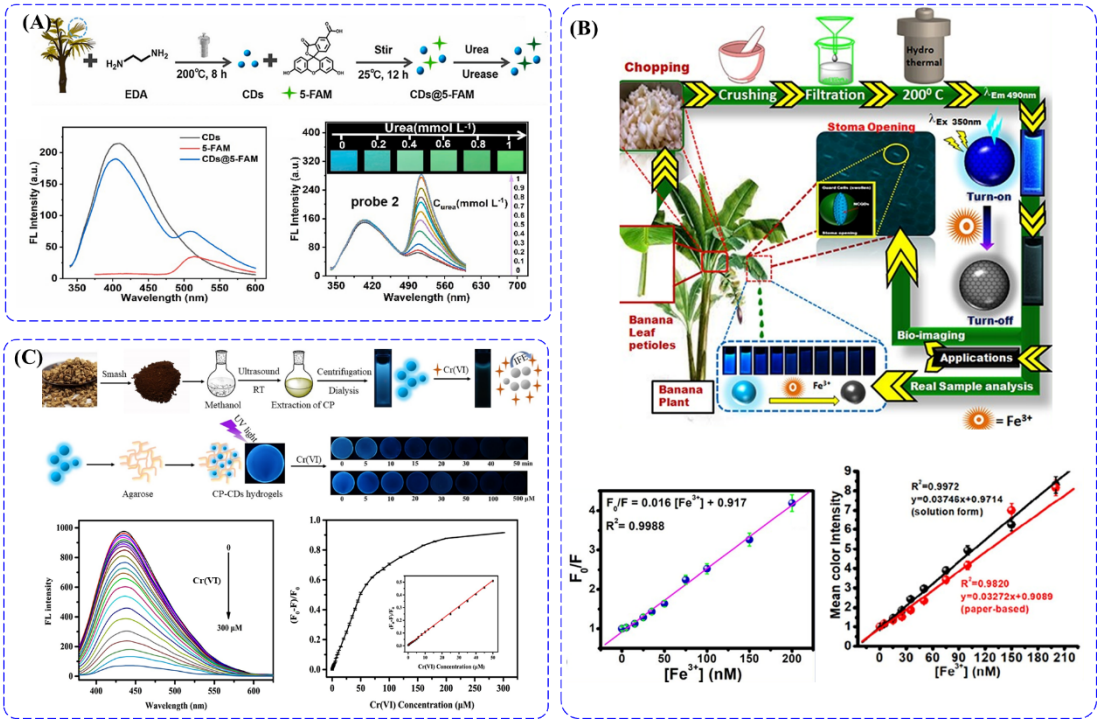

Figure 3. (A) The preparation process and fluorescence spectra of CDs@5-FAM, and the effect of increasing urea concentration on probe fluorescence spectrum. Reprinted with permission from Ref. (Bei et al., 2023). Copyright 2022, Elsevier. (B) Synthesis and fluorescence response of probe and construction of fluorescence sensing platform of smart phone. Reprinted with permission from Ref. (Korram et al., 2023). Copyright 2023, American Chemical Society. (C) Preparation of CP-CDs and detection of Cr (VI). Reprinted with permission from Ref. (Qiu et al., 2020). Copyright 2020, Elsevier.

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291 **Table 3.** Summary of precursors in biomass-derived carbon dots.

Carbon precursor	Quantum yield (%)	Size (nm)	Detector	Linear range	LOD	Recovery (%)	Ref.
Toona sinensis leaves	17	2.5	Urea	—	0.014 mmol·L^{-1} (CDs@5-FAM)	93	(Bei et al., 2023)
Andrographis paniculata	98.6–101.1; 97.6–103.4 (fluorescence spectrum)	170	Cu(II); L-cysteine	0.1–2 μM, 5–200 μM; 0.06–100 μM	71 nM; 12 nM (fluorescence spectrum)	98.2–101.6; 103.3–121.6 smartphone-integrated optosensors	(Zhang et al., 2022a)
Banana petioles	8.5	3.3	Fe^{3+}	5−200 nM	0.21 nM	98.7−105.4	(Korram et al., 2023)
Rice fried Codonopsis pilosula	12.8	9.60 (methanol), 11.54 (aqueous solution)	Cr (VI)	0.03−50 μM	15 nM	94.6–107.8	(Qiu et al., 2020)
Escherichia coli BW25113	15.8	2.9	p-NP, microbial cells	—	11 nM (p-NP)	—	(Qin et al., 2019)

CFS of L. acidophilus	—	2.8	Antimicrobial/ultraviolet protective bacterial nanocellulose film	—	—	—	(Koushehet al., 2020)
Chitosan	35	2.8	NO_2^-	1–500 μM	0.1 μM	—	(Sun et al., 2021)
Animal bones	—	6.7 (PBCDs); 6.6 (BBCDs); 9.5 (SBCDs)	Ag^+, Cu^{2+}, Hg^{2+}, Fe^{3+}, Pb^{2+}	—	—	—	(Fu et al., 2022)
Pork rib bones	—	4.2	Dimethoate	0.15-5.0 μM	0.064 μM	93-105	(Liu et al., 2020)
Cow milk	38	7	Sn^{2+}	—	17 μM	—	(Kumar et al., 2022)

3.3 Microorganism-based biomass

A variety of biomass materials have been used for carbon source research and exploration, including microbial materials. Carbon-containing substances can be obtained from microbial biomass, which provides raw materials for CDs preparation. Microbial cells are rich in organic compounds, including proteins containing N, S, and other heteroatoms, which are ideal materials for constructing CDs (Oladzadabbasabadi et al., 2023). Wei et al. prepared CDs using *Escherichia coli*

BW25113 (WT) as a carbon source, which can be used for microbial imaging and p-nitrophenol (p-NP) detection (Qin et al., 2019). It has good biocompatibility and can be excreted quickly through the intestine, which also proves that CDs have the characteristics of low toxicity. CDs can also be used as fluorescent probes for the selective detection of p-NP, and the monitoring mechanism is the internal filtration effect (**Fig. 4A**). Moradi et al. synthesized CDs with antibacterial activity from cell-free supernatant (CFS) of lactobacillus acidophilus (Kousheh et al., 2020). CDs were introduced into bacterial nanocellulose membranes, and functional nano-papers with anti-microbial/UV blocking capabilities were also prepared. The work by Moradi et al. was the first to apply the CFS of probiotics to CDs production. Xiong et al. used chitosan derived CDs for NO_2^- determination and bacterial imaging (Sun et al., 2021). Chitosan is a linear poly cationic biopolymer (Zhang et al., 2023c). The fluorescence of N-doped carbon dots (N-CDs) can be selectively quenched due to static quenching and internal filtration effect. Based on this finding, a fluorescence sensor can be designed to complete the measurement in a real sample. The prepared CDs have low toxicity to bacteria and can stain bacterial cells through cell membranes or cell walls, making them a good choice for biological imaging (**Fig. 4B**).

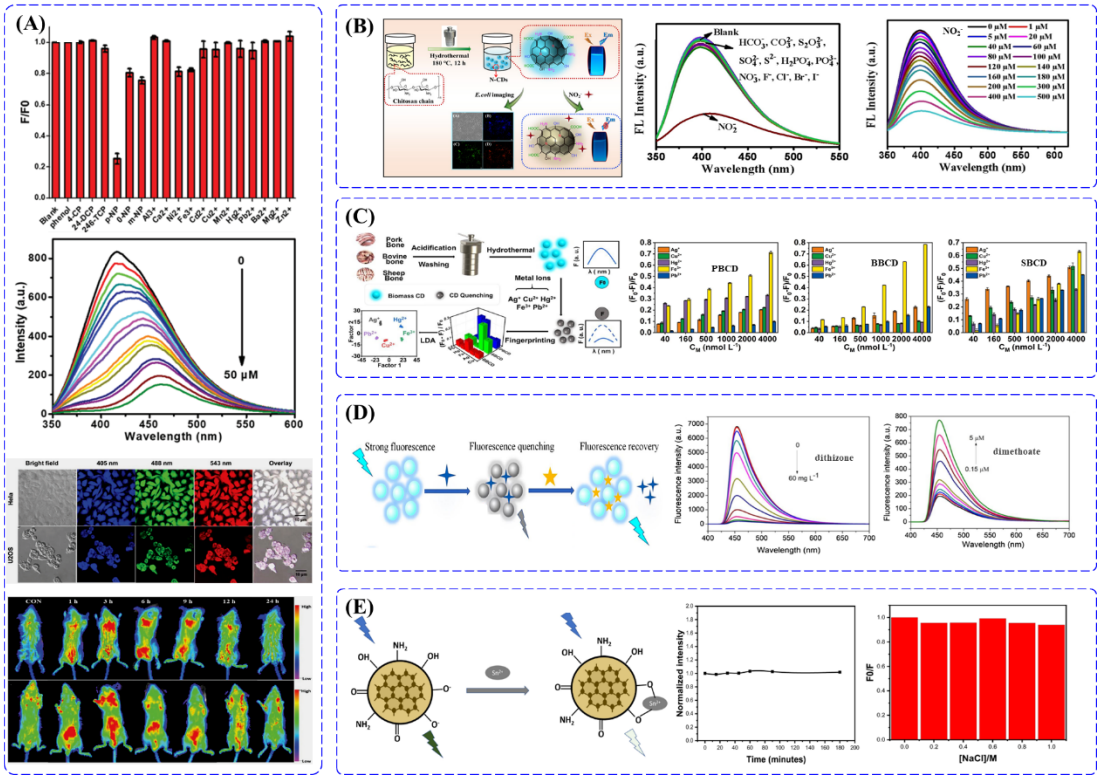

Figure 4. (A) Fluorescence quantization yield, confocal microscope images of CD-WTs reacting with different substances, and in vivo imaging of mice injected with CD-WTs. Reprinted with permission from Ref. (Qin et al., 2019). Copyright 2020, Royal Society of Chemistry. (B) Hydrothermal synthesis and application of N-CDs, and fluorescence emission spectra of various anions and different concentrations of NO_2^-. Reprinted with permission from Ref. (Sun et al., 2021). Copyright 2021, Elsevier. (C) Preparation of bone biomass CDs and their fluorescence response to metal ions. Reprinted with permission from Ref. (Fu et al., 2022). Copyright 2021, Elsevier. (D) The fluorescence switch mechanism and fluorescence spectra for biomass CDs. Reprinted with permission from Ref. (Liu et al., 2020). Copyright 2020, Elsevier. (E) The mechanism of CQDs luminescence quenching and light stability under ultraviolet irradiation and high salt conditions. Reprinted with permission from Ref. (Kumar et al., 2022). Copyright 2022, Springer Nature.

3.4 Animal remains-based biomass

Animal remains can also be explored as carbon sources, including bones (Fu et al., 2022; Liu et al., 2020), cow manure (Horst et al., 2021), fish scale (Dhandapani et al., 2020), and silkworm cocoons (Wang et al., 2020). The value of bone as kitchen waste is low, and the transformation into CDs improves its application value and realizes the reuse of waste resources. Chen et al. prepared pork bone BCDs (PBCDs), bovine bone BCDs (BBCDs), and sheep bone BCDs (SBCDs) via a hydrothermal method using different raw materials to distinguish heavy metal ions (Ag^{+}, Cu^{2+}, Hg^{2+}, Fe^{3+}, Pb^{2+}) (Fu et al., 2022). Due to the different content of elements in the three CDs, different affinities for metal ions led to different degrees of quenching (**Fig. 4C**). Ding et al. prepared CDs by treating pork rib bones in a simple way, which was cheap to manufacture and easy to operate (Liu et al., 2020). On this basis, the fluorescence switching process was also established. Dithizone was shown to quench the fluorescence of CDs, and the addition of dimethoate restored the fluorescence. Dithizone-functionalized CDs could be used for the detection of organophosphorus pesticide dimethoate, which was of great significance for the protection of the ecosystem (**Fig. 4D**). In addition to animal bones, CDs could also be created from cow milk through a simple hydrothermal synthesis process (Kumar et al., 2022). CDs can also selectively detect Sn^{2+} through a fluorescence quenching mechanism, which is an optional method to detect Sn^{2+} in the environment. What's more, CDs are stable under UV irradiation and high salt conditions, which also shows that they can be used in practical situations (**Fig. 4E**). The quantum yield of animal-derived CDs is lower than that of plant waste-derived CDs (de Oliveira and da Silva Abreu, 2021).

However, the animal material contains rich chitin and protein contents, and it contains rich C, O, N elements. N-containing groups give CDs special properties, such as those related to fluorescence (Kuang et al., 2023).

4. Effects of carbon dots for fluorescent sensing

4.1 pH

As previously stated, many factors affect the fluorescence color and intensity of CDs, such as pH (Das et al., 2020; Meierhofer et al., 2020), reactant ratio, temperature, and time of synthesis. Copper-doped fluorescent carbon dots (Cu-CDs) with blue fluorescence were prepared by Shuang et al. (Guo et al., 2021). Under acidic conditions, the fluorescence intensity decreases with the increase of pH value, which can be used to detect the change of intracellular pH value. Introducing the pH response of Cu-CDs into the study of fluorescent probes will further expand its application field (**Fig. 5A**). In addition, CDs prepared by Chen et al. with folic acid and nicotinic acid has a high sensitivity to pH, which is expected to be used for pH sensing (Gan et al., 2020). Using the change of zeta potentials, the protonation and deprotonation of CD surface functional groups were investigated to clarify the response of CDs in an acid-base environment (**Fig. 5B**). Zhang et al. synthesized orange luminescent carbon dots (O-CDs) using o-phenylenediamine and ethyl orthosilicate as raw materials using a hydrothermal method (Sun et al., 2022). O-CDs can be used as fluorescent ink to encrypt data. The fluorescence intensity is highest under neutral conditions. The surface functional groups undergo protonation and

deprotonation under acidic and alkaline conditions, with obvious fluorescence quenching. By utilizing the characteristics of O-CDs, anticounterfeiting ink is designed to be written on paper and then sprayed with hydrochloric acid to complete the encryption of information. Fluorescence is quenched by acid but can be restored by an alkaline solution. The O-CDs have potential application value in encrypted data and anticounterfeiting applications (**Fig. 5C**).

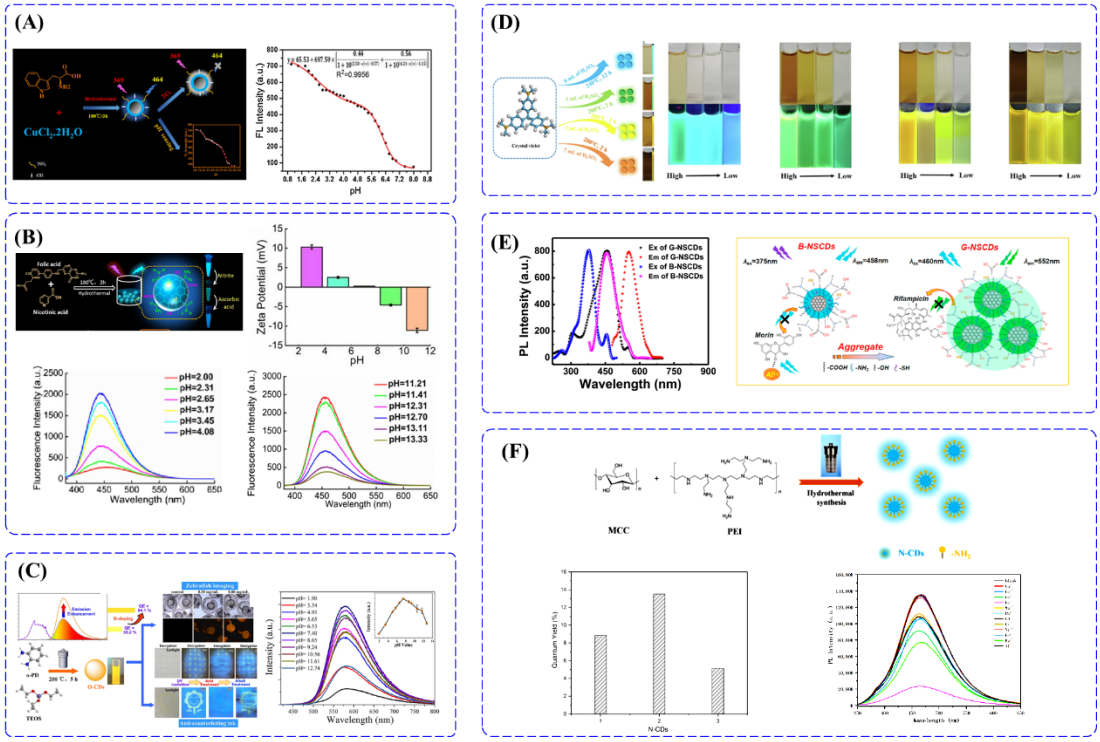

Figure 5. (A) The synthesis route of Cu-CDs and the fitting curve of relative fluorescence attenuation and pH. Reprinted with permission from Ref. (Guo et al., 2021). Copyright 2020, Elsevier. (B) CD composite diagram, CD correlation spectrum and zeta potentials at different pH levels. Reprinted with permission from Ref. (Gan et al., 2020). Copyright 2020, Elsevier. (C) The synthesis scheme of O-CDs and their application, as well as the luminescence spectra of O-CDs under different pH conditions. Reprinted with permission from Ref. (Sun et al., 2022). Copyright 2021, Elsevier. (D) Preparation of

four AIE-CDs and their AIE properties at different concentrations. Reprinted with permission from Ref. (Yang et al., 2022). Copyright 2022, Elsevier. (E) Excitation emission spectra of G-NSCDs and B-NSCDs, and schematic diagram of luminescence behavior regulated by the concentration of NSCDs. Reprinted with permission from Ref. (Tang et al., 2023). Copyright 2023, Elsevier. (F) Preparation of N-CDs and quantum yield of CDs. Reprinted with permission from Ref. (Fan et al., 2023). Copyright 2023, MDPI.

4.2 Analyte concentrations

By controlling the reactant ratio, it is possible to adjust the number of functional groups, the content of heteroatoms, and the degree of carbonization of CDs' surfaces (Su et al., 2020; Yang et al., 2022). Wang et al. prepared four kinds of fluorescent CDs using crystal violet and sulfuric acid solution as precursor and solvent, respectively (Yang et al., 2022). The different content of sulfuric acid solvent will affect the degree of carbonization of CDs to prepare different fluorescent colors of CDs. Sulfuric acid causes intermolecular and intramolecular oxidation dehydration to form large molecular compounds, and the emission wavelength is also redshifted. In their work, Wang et al. showed that by adjusting the solvent concentration, temperature, and reaction time, the luminous wavelength can be controlled, thus enabling applications in fields such as anticounterfeiting and cell imaging. The concentration of the analyte affects the emission wavelength of the CDs (**Fig. 5D**). The preparation of multicolor fluorescent CDs by controlling the fluorescence enables a new kind of fluorescent material with great potential (Cao et al., 2022a; Li and Gong, 2022; Yan et al., 2023). Chen et al. found that nitrogen-sulfur co-doped carbon dots (NSCDs) have green fluorescence at high concentrations (0.2 mg mL^{-1}) and blue

fluorescence at low concentrations (0.01 mg mL^{-1}) (Tang et al., 2023). It was further found that the detection behavior was also different under different concentrations (**Fig. 5E**). Fan et al. prepared nitrogen-doped carbon dots (N-CDs) using microcrystalline cellulose (MCC) and PEI as carbon and nitrogen sources (Fan et al., 2023). It was found that the ratio of MCC to PEI is an important factor affecting the fluorescence quantum yield of N-CDs. N-CDs with an MCC/PEI ratio of 1:1 have the highest quantum yield, and the N-CDs can selectively detect Fe^{3+}. (**Fig. 5F**).

4.3 Temperature

The fluorescence intensity or emission wavelength of CDs are affected by the temperature (Guo et al., 2020; Zhu et al., 2021). Zhang et al. prepared Cys-CDs with L-cysteine (Cys) and trisodium citrate dihydrate as raw materials, which showed a linear response of fluorescence intensity in the range of 10-70 ℃ (Guo et al., 2020). The fluorescence intensity decreases when the temperature rises and recovers when the temperature falls again. Quenching is attributed to the activation of nonradiative relaxation channels. Importantly, the light-emitting thermometer made by Cys-CDs can be reused (**Fig. 6A**). The fluorescence intensity of CDs prepared by Guan et al. is reversible at 25-95 ℃ (Zhang et al., 2020). The CDs use N-aminoethyl piperazine and citric acid as precursors. The reason for the decrease in fluorescence intensity with the increase in temperature is aggregation or the synergistic effect between oxygen-containing functional groups and hydrogen bonds. As a temperature sensor, it has the advantages of a wide response range and high sensitivity (**Fig. 6B**). The

yellow, fluorescent CDs prepared by Shai et al. using the hydrothermal method can be used to detect temperature (Chang et al., 2020). The fluorescence intensity varies with temperature and has a good linear relationship in the range of 25-40 ℃ and 45-80 ℃. The excellent response of CDs to temperature makes them suitable for use in temperature sensors (**Fig. 6C**).

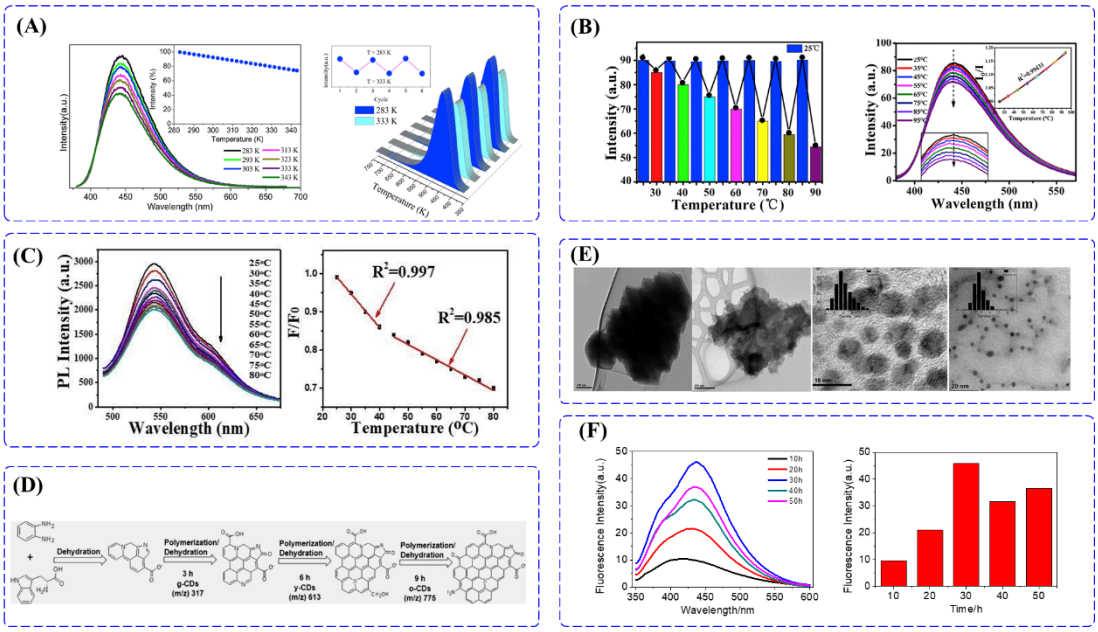

Figure 6. (A) Fluorescence spectra and temperature reversibility of Cys-CDs solution at different temperatures. Reprinted with permission from Ref. (Guo et al., 2020). Copyright 2019, Elsevier. (B) Temperature cycle test of CDs and emission spectra at different temperatures. Reprinted with permission from Ref. (Zhang et al., 2020). Copyright 2019, Elsevier. (C) y-CDs luminous intensity at different temperatures, and linearity of response between F/F_0 and temperature. Reprinted with permission from Ref. (Chang et al., 2020). Copyright 2020, the Royal Society of Chemistry. (D) Schematic diagram of MCDs formation. Reprinted with permission from Ref. (Shen et al., 2023). Copyright 2023, American Chemical Society. (E) TEM images and size distribution of CDs synthesized at different reaction times. Reprinted with permission from Ref. (Papaioannou et al., 2019). Copyright 2019, American Chemical Society. (F) Fluorescence spectra and histograms of Phe-CDs at

different reaction times. Reprinted with permission from Ref. (Pu et al., 2020). Copyright 2019, Elsevier.

4.4 Reaction time

In the synthesis process, the reaction time affects the fluorescence intensity and emission wavelength position of the fluorescent substance. Xu et al. synthesized multicolor CDs using o-phenylenediamine and L-tryptophan as precursors (Shen et al., 2023). With the increase in reaction time, the degree of graphitization and surface state changes. In this system, CDs form small sp^2 domains at 3 h. At 6 h, more precursors participated, and the CD sizes expanded. After 9 h, the CDs surface has extensive conjugated length. The blue, yellow, and orange CDs were prepared by controlling the reaction time, indicating that the reaction time plays an important role in the fluorescence of CDs (**Fig. 6D**). In the work of Titirici et al., CDs were synthesized from glucose by hydrothermal method (Papaioannou et al., 2019). Over the 2-12 h duration of the reaction process, the particle size decreases, and the crystallinity increases. In addition, the different reaction time also affects the intensity of fluorescence. CDs did not show fluorescence after a 2 h reaction. The generation of CDs fluorescence after 4 h could possibly be ascribed to the molecular fluorophore binding on the particle surface or embedding in the amorphous matrix (**Fig. 6E**). Ling et al. prepared CDs from phenylalanine and citric acid with blue fluorescence (Pu et al., 2020). The reaction time also has a certain effect on the fluorescence intensity of CDs. The luminous intensity of CDs increased gradually and reached the maximum at 30 h. The results showed that the fluorescence intensity of CDs was related to the

reaction time (**Fig. 6F**). Take hydrothermal method as an example: its temperature is roughly 180-200 ℃, and the time is 2-12 h. Carbonization at lower temperatures may require an increased reaction time, which can be shortened with the help of microwave-assisted synthesis (Ng et al., 2021). To obtain CDs with excellent performance, it is necessary to optimize the reaction time.

5. Improvements in CDs fluorescent sensing

5.1 Reaction condition optimization

CDs can be prepared by solvothermal method, with carbon source and solvent heated at high temperatures in an autoclave. The preparation process is convenient to operate, and the equipment is cheap (Hu et al., 2016; Kang et al., 2020). The fluorescence properties of CDs prepared at low temperatures are determined by the chromophore, whereas those at high temperatures are determined by the state of the carbonized core (Gao et al., 2021). Fan et al. prepared CDs using a solvothermal method with o-phenylenediamine and thiourea (Gao et al., 2021). Green emission CDs can be separated at relatively low temperatures, and green emission and red emission can be separated at temperatures higher than 170 ℃; high-temperature conditions make the fluorescence redshift. The reason for the red emission fluorescence characteristic produced at high temperatures is the polymer property, consisting of carbon core and intertwined polymer chains (**Fig. 7A**). However, the low-temperature CDs synthesis process is more consistent with the concept of green chemistry. Zhao et al. synthesized N,P co-doped CDs at low temperature (Zhao et al.,

2021). The preparation condition of N,P co-doped CDs is only 60 ℃, and the synthesis process takes only half an hour. In this synthetic system, the increase of temperature also causes the emission wavelength to redshift, whereas the fluorescence intensity decreases when the temperature exceeds 60 ℃ (**Fig. 7B**). The low temperature and short duration synthesis process of this system is closer to the concept of green chemistry. Compared with the high-temperature and long-duration CD synthesis process, the method of low-temperature CDs synthesis has more advantages. In the preparation process, the degree of carbonization of CDs is regulated by controlling temperature and time to obtain CDs with high quantum yield. Maity et al. used orange pomace to prepare CQDs by microwave technology (Kundu et al., 2023). During the preparation process, the filtered juice was heated in a microwave reactor for 10 minutes at 150℃ (300 W), and the impurities were filtered out to obtain brown CQDs (**Fig. 7C**). The synthesized CQDs have good detection effect on Cr^{6+} and 4-NP. Microwave-assisted synthesis is a simple and convenient method, whose reaction time can reach the order of minutes. In addition, to obtain high-quantum-yield CDs, bright and efficient fluorescent chromophores can also be introduced in the synthesis process, or CDs with uniform particle size prepared by template method (Zhang and Lu, 2023).

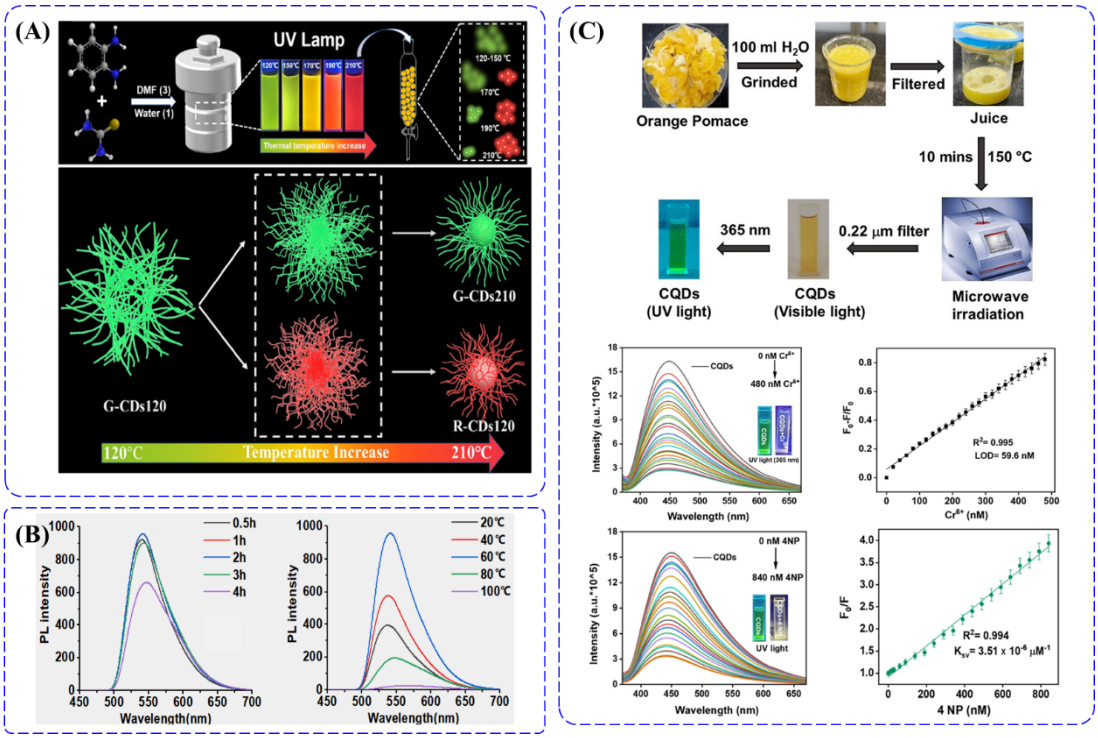

Figure 7. (A) Schematic diagram of preparation and possible growth mechanism of multicolor emission CDs. Reprinted with permission from Ref. (Gao et al., 2021). Copyright 2021, Elsevier. (B) Spectra of N,P-CDs at different reaction times and temperatures. Reprinted with permission from Ref. (Zhao et al., 2021). Copyright 2020, Elsevier. (C) Preparation and application of CQDs. Reprinted with permission from Ref. (Kundu et al., 2023). Copyright 2023, American Chemical Society.

5.2 Sustainable carbon sources

There are many kinds of CDs synthesis methods, and the production of CDs from green biomass materials is receiving increasing attention (Liu et al., 2019a). When using other carbon sources or nitrogen and sulfur doping to prepare CDs, it is difficult to avoid the shortcomings of expensive precursors and high costs. Fu et al. prepared thioctic acid-carbon dots (SCDs) using DL-thioctic acid as a sulfur source

and used SCDs as a probe to detect Hg^{2+} and thiophanate methyl (TM) (Wang et al., 2021a). The addition of Hg^{2+} can quench the fluorescence of SCDs at 438 nm, and TM can gradually restore the quenched fluorescence. The reason for quenching is the formation of a complex by static quenching, and the fluorescence recovery is the formation of a complex by TM and Hg^{2+}, which destroys the original structure. Biomass materials such as yeast powder (Cao et al., 2022b), rice fried codonopsis pilosula (Qiu et al., 2020), and cow milk (Kumar et al., 2022) can also be used as carbon sources to prepare CDs. Atchudan et al. used kiwifruit peel as a precursor to prepare CDs for Fe^{3+} detection (Atchudan et al., 2021). As a biomass material, kiwi fruit has rich functional groups. The use of chemicals can be reduced using plant materials. The CDs can be used as a sensor to detect metal ions in the human body or environment and have good selectivity and sensitivity. Li et al. prepared CDs from flax straw, which can be used for the detection of Co^{2+}, Cr^{6+}, and ascorbic acid (Hu et al., 2020). Flax straw contains cellulose, hemicellulose, and lignin, which is a good choice for preparing CDs. Cellulose is a promising sensing material because of its high mechanical strength and good degradation (Ma et al., 2023). The use of these biomass materials not only reduces the use of expensive and some toxic chemical reagents, but it also makes these materials reusable and more environmentally friendly.

5.3 Element doping strategy

CDs can be multifunctional by doping with heteroatoms (Guo et al., 2021). Doping of heteroatoms can adjust the optical properties or surface properties of CDs (Zhang et al., 2014). They can be doped with N (Ghafarloo et al., 2020; Latha et al., 2020; Zhi et al., 2022), P (Zhao et al., 2021), Cu (Guo et al., 2021), Fe (Alqahtani et al., 2023), S (Wu et al., 2023), and other heteroatoms (**Table 4**), thus expanding their application in substance detection and other fields. Shuang et al. prepared Cu-doped carbon dots (Cu-CDs) using L-tryptophan and copper chloride as precursors (Guo et al., 2021). By choosing quinine sulfate in water, the quantum yield of Cu-CDs is higher than that of undoped CDs. The fluorescence of Cu-CDs was quenched under the action of tetracycline antibiotics (TCs) based on internal filtration. Due to the doping of Cu on CDs, the strong attraction leads to the formation of Cu-CDs-TCs complexes. Chen et al. used nitrogen-doped CDs (N-CDs) as a probe for the detection of Fe^{3+} (Zhang et al., 2014). Nitrogen doping induces in CDs a strong blue fluorescence and high quantum yield. Importantly, the CDs preparation method is a solid phase thermal strategy, which is simple to operate and can control the transverse size and surface properties of N-CDs. Because of the strong electron affinity of N atoms doped in CDs, the emission wavelength appears as a blue shift compared with N-free CDs. The fluorescence intensity of N-CDs is higher, and the fluorescence lifetime is longer. In addition, Shakir et al. used N and S co-doped CDs (NS CDs) as a probe for the detection of Cr (VI), ascorbic acid and metallic yellow (Abbasi and Shakir, 2022). NS CDs are prepared with mesaconic acid, ethylenediamine, and sulfuric acid as C, N, and S sources. The quantum yield reached 33%. However, the

quantum yield of NCDs without sulfuric acid is only 13.8%. Element doping can adjust the electron energy level structure and chemical properties so that the function of CDs can be extended (Zhao et al., 2021). To improve the fluorescence sensing of CDs, the type of reaction solvent can be adjusted to modulate the spectral range covered by CDs (Liu et al., 2021a; Ran et al., 2023). CDs can also be used with molecularly imprinted polymers (MIPs) to improve the selectivity and sensitivity of the sensor (Ansari and Masoum, 2021). In addition, CDs are encapsulated in a porous material to reduce their accumulation of CDs and achieve an increase in optical properties (Li et al., 2021a).

Table 4. Heteroatom doping in carbon dots and their analytical performances.

Heteroatom doping	QY (%)		Emission wavelength (nm)		Detector	LOD	Ref.
	(undoped)	(doped)	(undoped)	(doped)			
Cu	2.1	9.5	—	464 (Ex 369)	Tetracyclines, pH	0.16 μM (Oxytetracycline)	(Guo et al., 2021)
N	9	31	442 (Ex 360)	435 (Ex 360)	Fe^{3+}	2.5 nM	(Zhang et al., 2014)
NS	13.8 (NCDs)	33 (NS CDs)	380 (Ex 320)	405 (Ex 350)	Cr (VI), ascorbic acid, metallic yellow	0.056 μM; 1 μM; 0.14 μM	(Abbasi and Shakir, 2022)

N,P	—	14	—	530 (Ex 430)	antibacterial agent, Sudan Red I	43 nM	(Zhao et al., 2021)
Fe/N	—	33.8	—	440 (Ex 365)	H_2O_2, glucose	0.36 μM (Glucose, fluorometric)	(Alqahtani et al., 2023)

Ex: excitation wavelength.

6. Future perspectives and challenges

Biomass-derived CDs are abundant, inexpensive, non-toxic, and have superior sensing capabilities. The analytical performance of biomass-derived CDs is similar to that of chemical-derived CDs, meaning that they meet the analytical requirements. Using animal derivatives, plants, and household waste as carbon sources can effectively improve the utilization rate of resources. At present, CDs using biomass as raw material have achieved great development. However, how to improve the preparation method and develop these CDs' potential application value is still a challenge. Biomass-derived CDs face the problem of low quantum yield, which needs to be solved by optimizing synthesis methods, element doping, and composite materials. Secondly, the purification of biomass-derived CDs is more challenging. Biomass-derived CDs are diverse and complex in composition, with many functional groups on the surface, and the surface composition and structure are unclear. In the

future, greater attention should be focused on purification technologies to treat or reduce by-products. In addition, the production of biomass-derived CDs is low and limited to laboratory research, making it difficult to achieve commercial model production and utilization. Finally, biomass-derived CDs are usually unstable at strong acidity, which limits their application in special environments.

Biomass-derived CDs have excellent optical properties and tunable fluorescence characteristics, which can be used as probes for analyte detection, fluorescent ink, and optical devices. CDs have broad application prospects in the manufacturing of light-emitting diodes, luminescent solar concentrators, and other optical devices. The application of CDs in luminescent solar concentrators lays a foundation for their practical application in the future. Their advantages such as low toxicity and good biocompatibility also expand the application of CDs in the biomedical field. In addition, CDs can be used in photocatalysis, electrode materials, room temperature phosphorescence materials, and in other fields. Given the development of the preparation and mechanism of CDs, their potential applications are expected to be extensive.

7. Conclusions

In this paper, various factors affecting the fluorescence detection performance of CDs are introduced, and the improvement of their preparation technology as a fluorescence sensing material is briefly described. Results have shown that the reactant concentration, reaction time, reaction temperature, and pH would affect the

application of CDs in fluorescence detection. New preparation technology, element doping, and other means can be used to expand its application prospects in different fields. In the fluorescence detection mechanism, the principle of aggregation-induced luminescence and the static quenching mechanism are briefly described. In addition, this paper introduces biomass material as a carbon source. Using plants, animal remains, and microorganisms as raw materials can not only reduce the preparation cost of CDs, but it can also increase the utilization efficiency of resources. The emergence of biomass-derived CDs enables researchers to discover many new possibilities: their excellent properties give them potential application value in fluorescence sensing, fluorescence ink, optical devices, biomedicine, photocatalysis, and other fields. A principal goal of this summary and review of biomass CDs is to stimulate further discussion and application in wider fields.

CRediT authorship contribution statement

Lili Yuan: Writing – original draft, Writing - review & editing, Conceptualization. **Congying Shao:** Writing – original draft, Writing - review & editing. **Qian Zhang:** Writing – original draft, Writing - review & editing. **Erin Webb:** Writing - review & editing. **Xianhui Zhao:** Writing - review & editing. **Shun Lu:** Writing – original draft, Writing - review & editing, Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests.

Data availability

No data was used for the research described in the article.

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