Coumarin-Modified Graphene Quantum Dots as a Sensing Platform for Multicomponent Detection and Its Applications in Fruits and Living Cells

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Cite This: ACS Omega 2020, 5, 7369−7378

ABSTRACT: In this work, coumarin derivatives (C) are used to enhance the fluorescence of graphene quantum dots (GQDs) by covalently linking the carboxyl groups on the edge of the GQD sheet. The as-synthesized coumarin-modified graphene quantum dots (C-GQDs) have a uniform particle size with an average diameter of 3.6 nm. Simultaneously, the C-GQDs have strong fluorescence emission, excellent photostability, and high fluorescence quantum yield. C-GQDs and CN\(^{-}\) can form a C-GQDs+CN\(^{-}\) system due to deprotonation and/or intermolecular interactions. The introduced hydroquinone (HQ) is oxidized to benzoquinone (BQ), and the interaction between BQ and the C-GQDs+CN\(^{-}\) system could lead to fluorescence enhancement of C-GQDs. Meanwhile, the redox reaction between BQ and ascorbic acid (AA) can be used for quantitative detection of AA with CN\(^{-}\) and HQ being used as substrates. Based on the above mechanism, C-GQDs are developed as a multicomponent detection and sensing platform, and the detection limits for CN\(^{-}\), HQ, and AA were 4.7, 2.2, and 2.2 nM, respectively. More importantly, satisfactory results were obtained when the platform was used to detect CN\(^{-}\), HQ, and AA in living cells and fresh fruits.

INTRODUCTION

The cyanide ion (CN\(^{-}\)) is a type of ion composed of a carbon-nitrogen triple bond, which is very toxic. CN\(^{-}\) can bind to iron ions in cytochrome, inhibiting the activity of enzymes, making organisms lose consciousness, and quickly leading to death.\(^{1,2}\) However, CN\(^{-}\) has important applications in producing plastics, mining gold, and other industrial materials.\(^{3}\) Hydroquinone (HQ), as a kind of phenolic organic compound, plays an important role in coal tar production, photographic imaging technology, papermaking, and other industrial fields.\(^{4−6}\) At the same time, it can cause extensive harm to human health and the environment.\(^{7−9}\) The biotoxicity of HQ is manifested in which a large amount of HQ is oxidized to benzoquinone (BQ), and the high dose of BQ is easily causing DNA double-strand breaks. Interestingly, ascorbic acid (AA) can inhibit the biotoxicity of BQ by reducing BQ to HQ.\(^{10,11}\) AA can also promote the formation of antibodies and the uptake of trace elements and increase thiaecomplex activity.\(^{12−14}\) Meanwhile, both too high and too low levels of AA in the blood can cause various diseases.\(^{15,16}\) Therefore, convenient, sensitive detection of these three targets is crucial in biological, clinic, environmental, and industrial fields.

At present, new strategies are quickly appearing for CN\(^{-}\), HQ, and AA detection including chromatography,\(^{17}\) capillary electrophoresis,\(^{18}\) colorimetry,\(^{19}\) and fluorescence probe. In these methods, the fluorescence probe has the advantages of simple and rapid operation, possibility for rapid and real-time detection, and high sensitivity. In recent years, the conventional organic small molecule and semiconductor quantum dot fluorescent probes have been greatly limited in practical applications due to their high toxicity, low biocompatibility, and low water solubility.\(^{20−24}\) As a new kind of nanomaterials, GQDs, including quantum constraint, size effect, and edge effect, also have the advantages of low toxicity, good water solubility, high biocompatibility, and ease of functionalization.\(^{25,26}\) It has attracted increasing interests in catalysis, medicine, bioimaging, and fluorescence-sensing applications.\(^{16,27,28}\)

Hence, we develop a functionalized C-GQD fluorescent material probe for exhibiting an excellent response of CN\(^{-}\), HQ, and AA. The graphene oxide is cut into quantum dots by a simple chemical oxidation method and then modified by a...
Scheme 1. Synthesis of C-GQDs

coumarin derivative (C) to get yellow powder with a uniform size. Significantly, its fluorescence quantum yield increased by 18%. We find that the fluorescence of C-GQDs is quenched in the presence of CN\(^-\) and then recovered after the addition of HQ. Finally, the fluorescence is quenched again after the addition of AA. This may be due to the deprotonation and/or intermolecular interactions between C-GQDs and CN\(^-\), as well as the fluorescence of the system is affected by redox.

Figure 1. (a) HRTEM and TEM images of C-GQDs, the number indicated was the lattice parameter; (b) the particle size distribution of C-GQDs; (c) AFM image of C-GQDs; (d) the corresponding height profile of C-GQDs; and (e) FT-IR spectra of GQDs and C-GQDs.
reactions between BQ and AA. Based on the above mechanism, a fluorescence sensing platform for multi-component detection of CN\(^{-}\), HQ, and AA is designed. In addition, C-GQDs can also be used for fluorescence response and imaging of CN\(^{-}\) and HQ in HeLa cells, and the C-GQDs +CN\(^{-}\)+HQ system can be used for AA detection in fresh fruits. As far as we know, it is the first time that a carbon material-based fluorescent sensor for "off–on" detection of CN\(^{-}\), HQ, and AA is used.

■ RESULTS AND DISCUSSION

Characterization of C-GQDs. Scheme 1 describes the preparation of C-GQDs. As shown in Figure 1a (TEM and HRTEM) and Figure 1b, C-GQDs were well distributed with an average diameter of 3.6 nm. In addition, the inset of Figure 1a shows that the lattice spacing of C-GQDs is 0.23 nm, which was consistent with the plane spacing of graphene (110), indicating that C-GQDs had a similar crystal structure with graphene.\(^{31}\) In addition, the AFM image of C-GQDs in Figure 1c,d indicated that their heights were less than 2.3 nm, which confirmed that the C-GQDs only contain 1–6 layers of graphene nanosheets. The above results confirmed that we obtained graphene quantum dots via simple chemical oxidation of graphene oxide.

Figure 1e presents the FT-IR spectra of the synthesized GQDs and C-GQDs. As we could see, the sharp absorption peak at 1620 cm\(^{-1}\) corresponds to the skeleton vibration of graphene, which indicated that the sp\(^2\) C of GQDs had not been completely destroyed during the preparation process, and the structure similar to graphene still exists.\(^{32}\) There was a broadband around 3396 and 1385 cm\(^{-1}\) corresponding to the stretching vibrations and deformation vibration of O–H groups. Comparing C-GQDs with GQDs, the peak at 1720 cm\(^{-1}\) (stretching vibrations of the carboxylic group on GQDs) disappeared and a new peak appeared at 1706 cm\(^{-1}\) (C-GQDs), which can be assigned to the amide bond (–CO–).
absence of CN groups, such as GQDs were successfully linked by an amide group and C-
results of FT-IR analysis indicated that the intermediate C and (Figure 2c), which were attributed to C
peaks appeared at 284.8 and 288.1 eV in C1s of C-GQDs C
C of GQDs (Figure 2b), we could derive three component peaks
at 284.4, 286, and 288.8 eV, which correspond to C
N and C
O bonds. Also, the N1s spectra of C-GQDs (Figure 2d),
were attributed to C
N and C
N—
C
O bonds. Also, the N1s spectra of C-GQDs (Figure 2d) could be deconvoluted into two component peaks with
binding energies at about 398.9 eV (N—C/N=O) and 401.2
eV (N—H). These results were consistent with FT-IR analysis,
Figure 3. I n Figure 3a, significant absorption peaks at around 450 nm for free C and C-GQDs were
observed and, in comparison, only a little bulge at 450 nm for
GQDs. In DMF/H2O (v/v = 1:1) solution, C-GQDs and C
displayed significant fluorescence intensities at 507 and 522
nm, respectively, when excited at 450 nm, and GQDs almost
had no obvious fluorescence intensity above 450 nm (Figure
3b). The above results indicated that the increase in
fluorescence intensity of GQDs was due to the successful
introduction of C. In addition, the quantum yield (QY) of C-
GQDs was 18% higher than that of bare GQDs when
rhodamine 101 in ethanol (QY = 95%) was used as the
reference, and the fluorescence enhancement could be ascribed to the successful modification of GQDs. As shown in Figure
S2, C-GQDs exhibits emission behavior independent of excitation, this could be associated to the intrinsic nature of
C. The content of intermediate C was measured to be 166.5
mg per 10000.0 mg of C-GQDs by the standard addition
experiments. Furthermore, as shown in Figure S3 within 4 h,
the individual fluorescence intensities remained fundamentally stable for both GQDs and C-GQDs, which illustrate that they
both have good photostabilities.

Fluorescent Detection of CN−. The gradual quenching of the
fluorescence intensities for C-GQDs was realized with the
different concentrations of CN− (0–1833 μM) incubation, and
the fluorescence quenching efficiency reached the maximum
when the concentration of CN− reached 1166.7 μM (Figure
4a). A good linear calibration graph of C-GQDs (R2 = 0.9929)
under the concentration of CN− in the range 10–90 μM was
measured, and the detection limit of 4.7 nM was received
according to a 3σ/k (σ is the standard deviation of 20 blank
signals, and k is the slope of the linear fit curve), which
indicates that C-GQDs can detect CN− lower than previous
reported (Table S1).

To further estimate the detection performance of C-GQDs
can detect CN−, a competition experiment was conducted (Figure
4b). Among anions, only CN− caused a significant fluorescence quenching response. After adding CN− (1166.7 μM) and
different anions (F−, Cl−, Br−, I−, HSO4−, H2PO4−, and AcO−
were 3000 μM) to C-GQD solution, the phenomena of fluorescence quenching for C-GQDs still retained in the
presence of an excess of analytes. From what had been observed above, C-GQDs had the ability to selectively
response to CN− with the significant fluorescence quenching
without disturbance caused by various analytes. The response
time test (Figure S4) showed that the quenching efficiency
reached the maximum within 5 min and maintained stable
fluorescence intensity during the test time. Therefore, C-
GQDs can detect CN− sensitively and in real time.

Fluorescent Detection of HQ. To evaluate the
fluorescence response of the C-GQDs+CN− system to HQ,
the response of the C-GQDs+CN− system to some HQ
analogues was studied in the same solvent system. The
fluorescence response of the C-GQDs+CN− system (C-GQDs,
30 mg/L; CN−, 600 μM) to some HQ analogues (resorcinol
(RC), catechol (CC), phenol, p-aminophenol, m-aminophenol,
phloroglucinol, glucose, and fructose were 333.3 μM) was
recorded (λex = 413 nm), and the results are given in Figure S5.

Figure 4. (a) Fluorescence spectra of C-GQDs (30 mg/L) in the presence of different concentrations of CN− (0–1833 μM) in DMF/H2O (v/v, 1:1) solution; inset: variation of fluorescence intensity as a function of the concentration of CN− (10–90 μM). (b) Selectivity of C-GQDs toward CN− over the other competing anions. The black bars represent the fluorescence response of C-GQDs to different anions (1166.7 μM) in the absence of CN−, and the red bars represent the fluorescence response of C-GQDs to different anions (3000 μM) in the presence of CN− (1166.7 μM); λex/em = 450/507 nm.
Interestingly, no obvious change on the fluorescence spectra was observed in the presence of the aforementioned HQ analogues. However, the fluorescence intensity of the C-GQDs + CN− system was evidently renewed after the addition of HQ and exhibited a blueshift. It was found by the following titration experiments that the successive addition of HQ (0−666.7 μM) into the C-GQDs+CN− system solution significantly led to a stepwise enhancement in fluorescence intensity of C-GQD solution (Figure 5a). When the concentration of HQ arrived at 333.3 μM, the fluorescence intensity was enhanced by 96-fold and resulted in a 41 nm blueshift of the maximum emission wavelength. Simultaneously, there was a good linear relationship in the concentration range of HQ from 63.3 to 100 μM, and the detection limit was 2.2 nM. This performance was superior to most of the reported detection methods (Table 1).

Table 1. Comparison of Different Biosensors for the Detection of HQ

| system                  | linear range (μM) | detection limit (μM) | ref. |
|-------------------------|-------------------|----------------------|------|
| fluorescent/N/S/P-CDs   | 0.56−375          | 0.160                | 4    |
| fluorescence/SiQDs      | 6−100             | 2.630                | 6    |
| fluorescence/carbon dots| 0.1−50            | 0.10                  | 7    |
| fluorescence/CPNs       | 0.01−50           | 0.005                | 9    |
| fluorescence/carbon dots| 0.1−10            | 0.020                | 11   |
| fluorescence/g-CNQDs/H2O2| 0.5−11.6         | 0.04                  | 44   |
| fluorescence/ C-GQDs-CN−| 63−100            | 0.002                | this work |

*aN/S/P-CDs: nitrogen/phosphorus/sulfur-codoped carbon dots. bSiQDs: silicon quantum dots. cCPNs: conjugated polymer nanoparticles. d*g-CNQDs/H2O2: graphite phase carbon nitride quantum dot.

To evaluate the competitive selectivity of the receptor in the C-GQDs+CN− system in the presence of HQ analogues, we studied the competitive experiment of C-GQDs+CN− system (Figure 5b). Among HQ analogues inspected, there was no significant effect on the fluorescence intensity of the C-GQDs + CN− system. Thus, the C-GQDs+CN− system had the characteristic of detecting HQ in the benzenediol compounds (HQ, CC, RC).

Fluorescent Detection of AA. Figure 6a shows the fluorescence quenching responses of the C-GQDs+CN−+HQ (C-GQDs, 30 mg/L; CN− 600 μmol/L; HQ, 333.3 μM) system toward variable concentrations of AA. These results showed that the fluorescence intensity of the C-GQDs+CN−+HQ system was quenched proportionately on the increasing AA concentration from 0 to 666.7 μM. When the titrated concentration of AA reached 600 μM, the fluorescence was almost quenched completely. As shown in the inset of Figure 6a, the quenching ratio is linear with respect to AA concentration (R2 = 0.9912), and the lower limit of detection of AA was assessed to be 2.2 nM. Comparing the method with the reported probes (Table S2), the results show that the method established in this paper has better analytical performance.

The selectivity and competitive experiments of the C-GQDs + CN−+HQ system were carried out using several potential interferences (citric acid (CA), cysteine (Cys), lysine (Lys), glycine (Gly), tyrosine (Tyr), glucose, fructose K+, Ca2+, Na+, Mg2+, HSO4−, H2PO4−) of AA, respectively. As shown in Figure 6b, only AA among all investigated targets caused fluorescence quenching of the C-GQDs+CN−+HQ system, which was hardly affected in coexistence of the potential interferences of AA. These results confirm that the C-GQDs+CN−+HQ system could specifically detect AA in the presence of multiple potential interferences.

Possible Sensing Mechanism. As shown in Figure 7a, after adding CN−, the emission of the C-GQDs was dramatically quenched. When AA appeared in the system, fluorescence intensity of GQDs did not change significantly. The fluorescence of the C-GQDs+CN− system could be effectively enhanced after the addition of HQ, and the fluorescence maxima emission wavelength (λem) exhibited a blueshift, while HQ did not have influence on the fluorescence of C-GQDs (Figure 7b). Interestingly, we found that the C-GQDs+CN−+HQ system was sensitive to AA, and their strong fluorescence was obviously quenched upon the addition of AA, while AA did not have influence on the fluorescence of the C-GQDs+CN− system. The above results confirmed the
proposed strategy of sequential “OFF−ON−OFF” detection of CN−, HQ, and AA for C-GQDs.

Based on the above strategy, we suggested the possible reaction mechanism of C-GQDs. The fluorescence quenching of C-GQDs might be ascribed to deprotonation and/or intermolecular interactions (hydrogen bonding and electrostatic interaction) between CN− and C-GQDs.39−41 As shown in Figure S6, a new UV−vis absorption peak at 413 nm appeared after HQ was added into the C-GQDs+CN− system, and the color of the system changed. Simultaneously, TEM images of the system (Figure S7) showed that a network complex with a larger particle size appeared after the addition of HQ, and the morphology was in sharp contrast with that of C-GQDs. These results indicated that HQ might be oxidized.
to BQ, then BQ and C-GQDs may formed a quinone-amine or n-π clathrate. It is worth mentioning that after the addition of AA, the UV−vis absorption peak of the C-GQDs +CN−+HQ system disappeared and the color recovered. Analysis of these results showed that BQ might be reduced to HQ by AA, which affected the interaction between BQ and the C-GQDs+CN− system and resulted in the fluorescence quenching. Combined with the fluorescence properties of C-GQDs, we suggested that the logical mechanism for detecting CN−, AA, and HQ might be as Scheme 2.

Analysis of AA in Real Samples. The method developed in this paper for detecting AA has good feasibility and can be applied to real sample analysis. Due to the lower detection limit of the method, the sample was pretreated to be subjected to large-fold dilution, which greatly reduced a possible interference. In the analyses of fruit and beverage ingredients, a good recovery rate was obtained (Table 2), which indicated that the method has certain practicability and can be used for the detection of real samples.

| samples | initial amount (mg/100 g) | standard added (μM) | standard detected (μM) | recovery (%) | RSD (%) |
|---------|-----------------------------|----------------------|------------------------|--------------|--------|
| kiwi    | 46.2                        | 30                   | 31.3                   | 104.3        | 1.9    |
|         | 50                          | 49.9                 | 99.8                   | 1.0          |        |
| lemon   | 27.1                        | 30                   | 29.6                   | 98.6         | 1.2    |
|         | 50                          | 50.2                 | 100.4                  | 1.1          |        |
| orange  | 29.2                        | 30                   | 31.1                   | 103.7        | 1.8    |
|         | 50                          | 50.9                 | 101.8                  | 0.8          |        |
| pineapple | 35.5                       | 30                   | 28.7                   | 95.7         | 2.2    |
|         | 50                          | 50.2                 | 100.4                  | 0.9          |        |
| juice drink | 6.5                 | 30                   | 30.3                   | 101.0        | 0.9    |
|         | 50                          | 50.1                 | 100.2                  | 1.3          |        |

**Bio-Imaging Studies.** Since C-GQDs had excellent detection performance for CN− and HQ, we evaluated its application in HeLa cell imaging. It was shown that the cell viability was assessed by an MTT assay in Figure S8. After incubating HeLa cells with C-GQDs (0−200 mg/L) for 24 h, the cell viability was about 86%, indicating that its cytotoxicity was extremely low. Therefore, C-GQDs could be used for the biological imaging in living cells. CN− (600 μM) and HQ (333.3 μM) solutions were introduced into C-GQD (30 mg/L) pretreated cells, and then the cells were treated by the standard procedure. As shown in Figure 8A,D,G, HeLa cells showed a stronger green fluorescence when incubated with C-GQDs. In the presence of CN−, C-GQD-stained cells would cause intracellular fluorescence quenching, indicating that C-GQDs have a high sensitivity for the detection of CN− (Figure 8B,E,H). It is as expected that the intracellular fluorescence intensity was recovered and showed stronger fluorescence intensity when the cells were further incubated with HQ, which is coordinated with the testing extracellular before (Figure 8C,F,I). The above results indicated that C-GQDs had low biological toxicity, good cell permeability, and sensitive to the fluorescence responses of CN− and HQ. This provided a new sensing platform for intracellular detection of CN− and HQ.

**CONCLUSIONS**

To conclude, a coumarin-functioned graphene quantum dot OFF−ON−OFF fluorescent probe (C-GQD) was developed for the detection of CN−, HQ, and AA in an aqueous system. The analysis of C-GQD results showed that it had been successfully modified by the coumarin derivative (C) and exhibited good dispersion, biocompatibility, and biolabeling potentials. The fluorescence spectra showed that the selective detection of CN− was achieved based on fluorescence quenching of C-GQDs, and its fluorescence could be restored.
and exhibited a blueshift after the addition of HQ that could be used to detect HQ with high sensitivity. Notably, the C-GQDs +CN−+HQ system could detect AA by fluorescence enhancement again. At the same time, C-GQDs could be successfully used to respond for AA in fresh fruits. Furthermore, it can be used to visualize exogenous CN− and HQ in HeLa cells by fluorescence turn-on signals. This work not only provides a new strategy to detect CN−, HQ, and AA but also provides a basis for functionalized graphene quantum dots as a new sensing platform.

**EXPERIMENTAL SECTION**

**Materials and Instrumentation.** Transmission electron microscopic (TEM) patterns were carried out with HITACHI H-7650 operated at 100 KV (America). A Tecnai G2 F30 high-resolution transmission electron microscope was used to investigate the morphologies of the C-GQDs (Japan). An atomic force microscope (AFM) was used to investigate the dimension of the C-GQDs (Germany). FT-IR measurements were performed by PerkinElmer Spectrum One (America). Thermo Fisher ESCALAB250Xi X-ray photoelectron spectroscopy (XPS) was carried out to detect the binding energies of all relative elements (America). A Puxi TU-1901 spectrophotometer was used for UV−vis absorption spectrum testing (China). A PerkinElmer LS55 fluorescence spectrophotometer was used for fluorescence spectrum testing (America). An Olympus IX51 biological inverted microscope was used for living cell imaging (Japan).

4-(N,N′-Diethylamino)salicylaldehyde was purchased from Chengyu Chemical Co., Ltd. in Beijing, China. Diethyl malonate, rhodamine 101, 1,3-dithioisocarbocyanide, hydrochloride (EDC), and 3-(maleimido)propionic acid N-hydroxysuccinimide ester (NHS) were purchased from Behringer Technology in America. H2SO4, HNO3, and H2O2 were purchased from Tianjin Kermle Chemical Reagent Co., Ltd. in Beijing, China. Diethyl amonium chloride (EDC), and 3-(maleimido)propionic acid N-hydroxysuccinimide ester (NHS) were purchased from Behringer Technology in America. H2SO4, HNO3, and H2O2 were purchased from Tianjin Kermle Chemical Reagent Co., Ltd. in Beijing, China. Hydroquinone, resorcinol (RC), catechol (CC), and α-ketoglutaric acid were purchased from Chengyu Chemical Co., Ltd. in Beijing, China. Hydroquinone, resorcinol (RC), catechol (CC), phenol, ρ-aminophenol, m-aminophenol, phloroglucinol, glucose, fructose, ascorbic acid (AA), cysteine (Cys), lysine (Lys), and tyrosine (Try) were purchased Tianjin Kermle Chemical Reagent Co., Ltd. in China. The cations were nitrates, and anions were tetrabutylammonium salts. The above reagents were of at least an analytical grade.

**Preparation of C-GQDs.** A Coumarin derivative (C) was prepared according to the literature. 30 The synthesis of C-GQDs was carried out according to the literature with a certain modification. In short, GQDs (50 mg) were blended with DMF (20 mL) and sonicated for 1 h to form homogeneous dispersion, EDC (76.8 mg, 0.4 mmol) was added into this system. Subsequently, 0.01 M HCl was added dropwise to the above solution until the pH of the system was 5. After vigorously stirring for 30 min at 25 °C, NHS (23.0 mg, 0.2 mmol) was added into the dispersion and 0.01 M NaOH was used to adjust the pH value of the system to 9, followed by activation for 3 h. C (69.2 mg, 0.2 mmol) was added into the above system, which was persistently stirred for 48 h at 25 °C. Finally, the reaction solution was dispersed to 100 mL water and repeatedly extracted by CH2Cl2. The extracted aqueous phase was further purified by a dialysis bag (1000 Da) for 48 h and freeze-dried to obtain C-GQDs (46 mg).

**Procedure for CN−, HQ, and AA Determination.** A 300 mg/L solution of C-GQDs was prepared in DMF/H2O (v/v = 1:1) for spectroscopic determination. The respective amounts of inorganic salts (KNO3, NaNO3, Ca(NO3)2, Mg(NO3)2) and organic molecules (HQ, RC, CC, phenol, ρ-aminophenol, m-aminophenol, phloroglucinol, glucose, fructose, AA, Cys, Lys, CA, G1, Tryr) were dissolved in water to afford the 0.1 M stock solutions. At the same time, it is necessary to configure tetrabutylammonium salts (F−, Cl−, Br−, I−, HSQ−, H2PO4−, AcO−, and CN−) as a 0.1 M anion stock solution. In a typical experiment of C-GQDs with targets, test solutions were prepared by placing 0.5 mL of C-GQD solution into a 5 mL test bottle, adding the different target stock solutions, and then diluting the solution to 5 mL with DMF and H2O (v/v = 1:1). Spectral data were recorded after 12 h, and the resulting solution was shaken well before collecting fluorescence data. Simultaneously, we needed to perform three time parallel experiments for received reliable data.

**Real Sample Measurement.** The real sample needed to be pretreated before testing. First, fresh fruits were homogenized by using a commercial blender and then centrifugation in a centrifuge at 8000 rpm for 15 min. Subsequently, these samples containing AA (fresh fruit (pineapple or something) and juice drinks) were first filtered by a 0.22 μm microporous filtration membrane and diluted to an appropriate concentration in the detection range with distilled water. Test solutions were prepared by placing 0.5 mL of C-GQDs (300 mg/L) into a 5 mL volumetric flask and diluting the solution with real samples and DMF (v/v = 1:1) to the corresponding concentration. Finally, the analyte and AA real sample were added to the test bottle, and the fluorescence spectra were recorded at room temperature.

HeLa cells were maintained in an incubator at 37 °C, 5% CO2 environment, feeding with 10% FBS (fetal bovine serum). The cells were cultured in a 96-well plate in culture media for cytotoxicity tests. Then, the C-GQDs at different concentrations (0–200 mg/L) were added into the cells for further incubation at 37 °C for over 24 h. To further determine the survival rate of HeLa cells, a 0.5 mg/mL MTT medium was added to the cells for about 12 h, and then the resulting formazan crystals were dissolved in DMSO (0.1 mL) and counted using a SpectraMax M2e microplate reader. For intracellular CN− and HQ imaging, cultured HeLa cells were incubated with 30 mg/L C-GQDs for 4 h and washed with mixed phosphate, added CN− (600 μM) was incubated for 8 h, and then added HQ (333.3 μM) was incubated for 8 h and washed again with mixed phosphate. Finally, the confocal microscopy images for three conditions were observed on a laser scanning confocal microscope, respectively.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b04387.

Absorbance intensity of C-GQDs in the presence of different concentrations of coumarin (Figure S1), their fluorescence spectra at different excitation wavelengths (Figure S2), and their spectra change within 4 h (Figure S3) under ultraviolet light; comparison of different biosensors for the detection of CN− and AA (Tables S1 and S2); effect of reaction time of C-GQDs+CN− system (Figure S4); fluorescence spectra of C-GQDs
+CN⁻ system in various analogues of HQ (Figure S5); UV spectra of C-GQDs after adding different analytes (Figure S6); TEM images of the C-GQDs+CN⁻+HQ system (Figure S7); and cell toxicity test of C-GQDs (Figure S8) [PDF]

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the support by the Qiqihar University Graduate Innovation Fund Grants (YJSCX2018-031X), the Fundamental Research Funds in Heilongjiang Provincial Universities (135209201), and the Province Natural Science Foundation of Heilongjiang (B2015017).

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