Carbon Quantum Dot-Incorporated Chitosan Hydrogel for Selective Sensing of Hg$^{2+}$ Ions: Synthesis, Characterization, and Density Functional Theory Calculation

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ABSTRACT: A carbon quantum dot-based chitosan hydrogel was prepared in this work as a fluorescence sensor for the selective sensing of Hg$^{2+}$ ions. Among the eight tested metal ions, the prepared hydrogel exhibited remarkable sensing selectivity and sensitivity toward Hg$^{2+}$. The results demonstrated that a prominent fluorescence quenching at 450 nm was observed in the presence of Hg$^{2+}$ with a linear response range of 0−100.0 nM and an estimated limit of detection of 9.07 nM. The as-prepared hydrogel demonstrates pH-dependent fluorescence intensity and sensitivity. The highest fluorescence intensity and sensitivity were obtained under pH 5.0. The excellent sensing selectivity could be attributed to a strong interaction between the hydrogel film and Hg$^{2+}$ ions to form complexes, which provokes an effective electron transfer for fluorescence quenching. Results from density functional theory (DFT) calculation confirm that the interaction energies (ΔIE) of the hydrogel with three toxic metal ions (Hg$^{2+}$, Cd$^{2+}$, and Pb$^{2+}$) are in the following order: Hg$^{2+}$ > Cd$^{2+}$ > Pb$^{2+}$.

INTRODUCTION

Environmental contamination by heavy metals has been a serious threat to the ecological system and human health due to their increasing use in domestic, industrial, agricultural, and medical applications.1 Non-biodegradable heavy metal ions have been extensively detected in various environmental matrices (water, wastewater, sediments, and biota). Rapid detection of toxic heavy metal ions in water plays an important role in the proper control of water quality and safety. To date, several detection methods, such as atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry, and inductively coupled plasma mass spectrometry, have been widely used for quantitative detection of heavy metal ions with high sensitivity.1 However, these techniques normally require complicated instrumental settings and pretreatment of water samples, which made them not feasible for on-site detection. There is a huge demand for developing rapid, economical, and highly selective on-site detection of toxic heavy metals.

Advances in fluorescent nanomaterials have paved the way for exploring new tools for optical sensing.3,4 Fluorescence detection (via the change of fluorescence intensity) exhibits the advantages of intrinsic simplicity, high selectivity, ultra-sensitivity, and low cost.4,5 Recently, carbon quantum dots (CQDs) with superior fluorescence properties, particularly those obtained from low-cost and abundant biomasses, have received great attention as fluorescent probes for the detection of heavy metal ions.6−11 However, bare CQDs tend to aggregate under neutral conditions, leading to a dramatic decrease in fluorescence intensity.12 To stabilize CQDs and
improve their performance, immobilization of CQDs within supramolecular frameworks has been pursued intensively in recent years.\(^{5,14}\) The solid matrices help control the assembly shape, interparticle distance, size, and porosity and provide versatile advantages of improved mechanical strength, chemical, and optical stability for CQDs. Through electrostatic interactions, covalent bonds, noncovalent bonds, or hydrogen bonds, CQDs can be incorporated into different solid matrices to prevent aggregation-induced fluorescence quenching.\(^{2,15}\)

Compared to other nanocarriers, hydrogels possess unique physical properties such as hydrophilicity, porosity, tunable cross-linking density, and stability in biological milieu. Therefore, embedding CQDs into polymeric hydrogels is a valuable technique to fabricate fluorescent composite materials with distinct characteristics and unique properties, which endow them with great potential applications such as sensors,\(^{16,17}\) optical and electrical devices,\(^{18}\) or imaging agents.\(^{19}\) So far, different assembly methods have been exploited to integrate CQDs into hydrogel networks. Sophisticated variations in structural architectures of composite hydrogel materials are made possible by host–guest interaction,\(^{20}\) incorporation of fluorophores into polymer matrices by infusion,\(^{21}\) self-assembly,\(^{22}\) and co-assembly of gel hybrids via bioconjugation.\(^{23}\)

A common fabrication strategy for fluorescent hydrogels is incorporating fluorophore entities (e.g., CQDs) into the hydrogel matrix by weak, noncovalent interactions.\(^{24}\) Novel chitosan–CQDs nanocomposite hydrogel films were recently prepared based on the electrostatic interaction of the positive charge on chitosan and the negative charge on CQDs.\(^{25}\) The electrostatic interaction endowed the as-prepared chitosan–CQDs films with excellent thermal stability, high tensile strength, and superior UV–Vis blocking properties. A similar method was employed to synthesize CQDs rooted agarose hydrogel films that can be used as both a colorimetric-optical sensor and membrane filter for a quartz of heavy metal ions.\(^{16}\) Although simple and easy to scale up, such noncovalent bonding of CQDs with the gel network may later result in leaching of the fluorescent entities and/or aggregation of CQDs, leading to the loss of luminescence. Further research is needed to investigate the chemical and fluorescence stability of the CQDs-incorporated hydrogels.

Selection of appropriate polymer matrices is not a trivial task for the design of a cost-effective CQDs-based composite hydrogel. Although both natural and synthetic polymers can be used to fabricate the CQDs-incorporated composite hydrogel, natural polymers in general offer good properties (e.g., nontoxicity, biodegradability, and biocompatibility) and are available in abundance.\(^{25}\) Chitosan, a biological cationic polymer composed of randomly distributed β-(1 → 4)-linked D-glucosamine and N-acetyl-D-glucosamine, has attracted great interest as a carrier for biomedical applications. Chitosan can be easily modified or blended with specific molecules to introduce distinct functionalities and/or properties (luminescence, conductivity, mechanical performance, etc.).\(^{26}\) Even though various chitosan hydrogels or chitosan hydrogel composites have been fabricated as adsorbents,\(^{27}\) food packing materials,\(^{28}\) wound dressing materials,\(^{29}\) and drug delivery carriers,\(^{30}\) few studies refer to the natural biopolymer-based fluorescence sensors for the detection of heavy metal ions. Therefore, incorporating fluorescence CQDs into chitosan hydrogels for constructing an effective fluorescence sensor deserves more research and development efforts.

In this study, a CQDs-based chitosan hydrogel film was fabricated as a fluorescence sensor for a rapid and selective detection of Hg\(^{2+}\), one of the most toxic metal ions, which may cause severe damage to the central nervous system and kidney failure even at a low exposure.\(^{31}\) It was anticipated that various binding groups on chitosan backbone help to enhance the sensing selectivity of the as-prepared hydrogel to Hg\(^{2+}\). The hydrothermal method was used to prepare nitrogen-doped CQDs (N-CQDs), which were subsequently embedded into the cross-linked chitosan matrices by ultrasonic agitation. Systematic characterization of the composite hydrogel film was performed to identify its structural and functional attributes. Quantitative fluorescence measurement of the composite hydrogel in metal solutions was carried out to evaluate its sensing performance. Finally, the selective sensing mechanism of Hg\(^{2+}\) by the composite hydrogel was elucidated with the aid of XPS analysis of the composite hydrogel. To complement the experimental results for the selective sensing of Hg\(^{2+}\) ion, we investigated the interaction between a single unit of the as-prepared hydrogel with three toxic metal ions (Hg\(^{2+}\), Cd\(^{2+}\), and Pb\(^{2+}\)) by a density functional theory (DFT) study.

### RESULTS AND DISCUSSION

#### Characterization of the N-CQDs@CTS Hydrogel Film

The FTIR spectra of the N-CQDs, GA cross-linked chitosan (GACTS), and N-CQDs-incorporated chitosan (N-CQDs@GACTS) hydrogel films were examined to validate the potential interactions between N-CQDs and the GACTS hydrogel film (Figure 1a). A broad peak at 3245 cm\(^{-1}\) is observed from the IR spectrum of the N-CQDs, which is due to the coalescing effect of the stretching vibrations of O–H, N–H, and C==C–H. Characteristic peaks at 1696, 1647, and 1532 cm\(^{-1}\) are attributed to the stretching vibrations of carboxylic C==O, C==N, and C==C in the aromatic rings of the N-CQDs, respectively.\(^{32}\) The IR band of N-CQDs at 1211 cm\(^{-1}\) can be ascribed to the stretching vibration of C–N. Characteristic IR bands of the GACTS film include the –NH bending vibration peaks at 1641.3 and 1552.5 cm\(^{-1}\) and the C–N and C–O stretching vibration peaks at 1150.8 and 1066.6 cm\(^{-1}\).\(^{27}\) After incorporating the N-CQDs into the GACTS hydrogel, the band peak of the C–N stretching vibration in the spectrum of the N-CQDs@GACTS hydrogel film is shifted to 1151.7 cm\(^{-1}\) from 1150.8 cm\(^{-1}\) due to the coalescence of the C–N stretching vibrations of both GACTS and N-CQDs. The IR results of the GACTS and the N-CQDs@GACTS do not support the formation of any primary bonds between N-CQDs and GACTS, which indicates that the N-CQDs were embedded into the GACTS matrix mainly through weak hydrogen bonds or electrostatic attraction.

XPS was employed to determine the surface composition of the N-CQDs, GACTS, and N-CQDs@GACTS hydrogel films, with the XPS survey spectra and atomic compositions being illustrated in Figure 1b. Binding energy (BE) peaks for C 1s, N 1s, and O 1s are observed from all the XPS spectra, and a fairly high percentage of nitrogen (15.84%) is present in the N-CQDs compared with those in the GACTS (5.17%) and the N-CQDs@GACTS (5.34%) hydrogel films.

#### Optical Properties of the N-CQDs@GACTS Film

Results from UV–Vis and fluorescence spectroscopies reveal that both the GACTS and N-CQDs exhibit blue fluorescence emissions. Figure 2 compares the fluorescence excitation and emission spectra of the GACTS and the N-CQDs@GACTS hydrogel films. As seen from Figure 2a, the
GACTS film can be excited by wavelengths between 240 and 250 nm. With the excitation wavelength being set at 250 nm, fluorescence emission peaks centered at 350 and 450 nm can be clearly observed. From the spectra of the N-CQDs@GACTS hydrogel film displayed in Figure 2b, the strongest excitation wavelength at 345−350 nm is observed, whereas its strongest emission wavelength is found to be 450 nm (λem = 450 nm). However, the fluorescence emission intensity of the N-CQDs@GACTS hydrogel film is about 25 times stronger than that of the GACTS film, which indicates that the fluorescence property of the composite hydrogel film mainly stems from the N-CQDs.

**Sensing Selectivity of the N-CQDs@GACTS Hydrogel.**

The sensing selectivity of the as-prepared N-CQDs@GACTS hydrogel toward Hg²⁺ was studied by mixing the N-CQDs@GACTS hydrogel with individual metal ion solutions at two concentration levels (100.0 and 1.0 × 10⁵ nM). As can be observed from Figure 3a, significant fluorescence quenching of the N-CQDs@GACTS hydrogel occurred after being immersed in 100.0 nM Hg²⁺ solution, leading to more than 20% reduction in its fluorescence intensity. Slight fluorescence quenching (<7.0%) of the N-CQDs@GACTS hydrogel by Pb²⁺ ion was also observed. Nonetheless, no significant quenching effects on the N-CQDs@GACTS hydrogel were observed by Cd²⁺, Zn²⁺, Cu²⁺, Fe³⁺, Ca²⁺, and Na⁺ solutions with the same concentration (100.0 nM). When the metal concentration was increased to 1.0 × 10⁵ nM (Figure 3b), significant fluorescence quenching by Fe³⁺ was detected. However, the magnitude of the quenching effect (1 − I/I₀) by Fe³⁺ (27.0 ± 1.1%) is approximately one-third of that by Hg²⁺ (85.0 ± 1.6%).

The quenching effect of the N-CQDs@GACTS hydrogel by Hg²⁺ in the presence of co-existing metal ions was also studied. A remarkable quenching effect of the hydrogel was induced by the mixed metal solution with Hg²⁺. Meanwhile, other coexisting interference ions showed negligible influences on the fluorescence response of the N-CQDs@GACTS hydrogel (Figure 3c). Results from Figure 3c clearly show that the N-CQDs@GACTS hydrogel is able to detect Hg²⁺ in the presence of possible interference cations (the concentration of each metal ion is 1.25 × 10⁴ nM).

**Fluorescence Sensing of Hg²⁺ Ions.** For a sensitivity study, the fluorescence response of the N-CQDs@GACTS hydrogel to different concentrations of Hg²⁺ was investigated and the results are demonstrated in Figure 4a. It can be seen that the fluorescence intensity of the N-CQDs@GACTS hydrogel keeps on decreasing with the increase in Hg²⁺ concentration, i.e., the degree of fluorescence quenching increases synchronously with the Hg²⁺ concentration. Figure 4b indicates the relationship between fluorescence response and the Hg²⁺ concentration. The ratios of I₀/I have a good linear correlation with Hg²⁺ concentration over a range of 0−

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**Figure 1.** Results of (a) FTIR and (b) XPS survey spectra of the N-CQDs, GACTS, and N-CQDs@GACTS films.

**Figure 2.** Excitation and emission spectra of (a) the GACTS and (b) the N-CQDs@GACTS films (DI water as a solvent).
The fluorescence quenching of the hydrogel by Hg$^{2+}$ ions can be described by the Stern–Volmer equation \[ I_0/I = 1 + K_{SV}c_M \] (1)

where $I_0$ and $I$ are the fluorescence intensity of the N-CQDs@GACTS hydrogel at 450 nm in the absence and presence of Hg$^{2+}$, respectively, $K_{SV}$ is the Stern–Volmer quenching constant, and $c_M$ is the Hg$^{2+}$ concentration. The $K_{SV}$ value is calculated to be 2.58 $\times$ 10$^8$ M$^{-1}$ with a correlation coefficient, $R^2$, of 0.998. The limit of detection (LOD) is estimated to be 9.07 nM with a signal-to-noise ratio of 3, which is competitive to the reported results by other CQDs-based optical sensors (Table 1).

The binding constant ($K_a$) of Hg$^{2+}$ ions to the N-CQDs@GACTS hydrogel could be determined using the below equation\textsuperscript{34,35}

\[ \frac{1}{\Delta I} = \frac{1}{\Delta I_{\max}} + \frac{1}{\Delta I_{\max}K_a} \left( \frac{1}{c_M} \right) \] (2)

In eq 2, $\Delta I$ ($I_0 - I$) is the change of fluorescence intensity of the N-CQDs@GACTS hydrogel in the presence of Hg$^{2+}$; $\Delta I_{\max}$ is the maximal change of fluorescence intensity; and $c_M$ is the concentration of Hg$^{2+}$. By linear regression of the $1/\Delta I$ versus $1/c_M$ curve based on eq 3 over a $c_M$ range of 1.0.0$–$1.0 $\times$ 10$^5$ nM, a $K_a$ value of 9.48 $\times$ 10$^4$ M$^{-1}$ has been obtained, which indicates extremely strong binding affinity between Hg$^{2+}$ and the N-CQDs@GACTS hydrogel.

The response time for fluorescence quenching is another important parameter to evaluate the fluorescence detection performance of fluorescent probes. Figure 4c shows the time-dependent fluorescence intensity ($\lambda_{em} = 450$ nm) of the N-CQDs@GACTS hydrogel quenched by 100.0 nM Hg$^{2+}$ solution excited at 350 nm. It can be seen that the fluorescence intensity of the N-CQDs@GACTS hydrogel remained almost unchanged after being immersed in the Hg$^{2+}$ solution for 30 min, which indicates that the quenching effect of the prepared composite hydrogel film to Hg$^{2+}$ is extremely fast and stable. It is believed that the fast mass transfer of Hg$^{2+}$ ions in the porous N-CQDs@GACTS hydrogel enables the full utilization of the high binding capacity of the chitosan hydrogel for Hg$^{2+}$ ions, resulting in the rapid response of fluorescence quenching. The digital photo in Figure 4c intuitively shows that the N-CQDs@GACTS hydrogel underwent fluorescence quenching in the presence of Hg$^{2+}$ ions under 395 nm UV light irradiation.

The influence of pH on the fluorescence intensity of the N-CQDs@GACTS hydrogel in the absence and presence of Hg$^{2+}$ ions was studied at $\lambda_{em}$ of 350 nm, and the results are illustrated in Figure 4d. In the absence of Hg$^{2+}$ ions, no significant variations in fluorescence intensity were observed when pH varies from 5.0 to 9.0. However, the fluorescence intensity of the hydrogel decreased significantly at lower pH levels (pH $\leq$ 3.0). In addition, the quenching effect caused by Hg$^{2+}$ ions also changed with pH. At pH 2.0, Hg$^{2+}$ ions were repulsed by the positively charged surface of the N-CQDs@GACTS hydrogel, leading to unobservable quenching effect.\textsuperscript{7} With the increase in pH, the surface of the N-CQDs@GACTS hydrogel becomes neutral or even negatively charged, and adsorption of Hg$^{2+}$ by the hydrogel is thus increased, resulting in an enhanced quenching effect. Nonetheless, Hg$^{2+}$ ions are unstable under basic pH, and the fluorescence quenching of the hydrogel by Hg$^{2+}$ remains unchanged at pH $\geq$ 7.0. The highest fluorescence quenching effect was observed at pH 5.0 (Figure 4d). In this study, the pH of the as-prepared N-CQDs@GACTS hydrogel is $\sim$ 5.2. It is unnecessary to further adjust the pH of the N-CQDs@GACTS hydrogel for Hg$^{2+}$ sensing.

**Detection of Hg$^{2+}$ in Tap Water.** To evaluate the capability of the N-CQDs@GACTS hydrogel for detecting Hg$^{2+}$ ions in real water samples, we spiked tap water samples with different Hg$^{2+}$ concentrations (100.0$–$1.0 $\times$ 10$^5$ nM). Figure 5 compares the fluorescence quenching of the hydrogel by Hg$^{2+}$ ions present in DI and tap water at the same concentrations. No fluorescence quenching occurred after the
N-CQDs@GACTS hydrogel was immersed in blank DI or blank tap water. However, the detection sensitivity of the hydrogel toward Hg²⁺ was significantly lowered (indicated by the recovery of the fluorescence of the hydrogel−Hg²⁺ complex) due to the presence of bacteria in tap water. Bacteria and other microorganisms in tap water contain amino acids, which are capable of disassociating the hydrogel−Hg²⁺ complex due to their strong Hg²⁺ binding affinity, resulting in the diminished quenching effect at a low Hg²⁺ concentration (100.0 nM). Such an effect is consistent with those reported for the “off–on” fluorescence detection of amino acids by CQD−Hg²⁺ systems.  

The calibration curve was observed over the entire Hg²⁺ concentration range. The recovery rates of the spiked tap water samples at four other concentrations of Hg²⁺ were determined and are listed in Table 2.

**Sensing Mechanism.** Most of the sensing mechanisms are rooted in noncovalent interactions between the hydrogel

| spiked concentration, nM | detected concentration, nM | recovery, % |
|--------------------------|---------------------------|-------------|
| 1.5 × 10⁴                 | 1.57 × 10⁴                 | 104.7       |
| 2.0 × 10⁴                 | 2.11 × 10⁴                 | 105.8       |
| 3.0 × 10⁴                 | 3.01 × 10⁴                 | 100.4       |
| 6.0 × 10⁴                 | 5.88 × 10⁴                 | 98.0        |
network and external stimuli. Compared with all the other tested metal ions, Hg\(^{2+}\) has the strongest oxidizing ability and it is therefore more easily combined with polar groups (−COOH, −OH, −NH\(_2\), etc.) on the surface of the N-CQDs@GACTS hydrogel by either chelation or electrostatic attraction. The formation of nonfluorescent ground-state complexes alters the absorbance of N-CQDs and accelerates the nonradiative electron transfer from the N-CQDs@GACTS hydrogel to Hg\(^{2+}\) ions, resulting in fluorescence quenching of the composite hydrogel. As such, static quenching contributes to the selective sensing mechanism of the N-CQDs@GACTS hydrogel to Hg\(^{2+}\) ions. In addition, the nonlinear Stern–Volmer plot illustrated in Figure 4b indicated that there are inaccessible fluorophores in the N-CQDs@GACTS hydrogel system.

The interaction between Hg\(^{2+}\) and the N-CQDs@GACTS hydrogel film was characterized by XPS analysis. Figure 6 exhibits the high-resolution and the deconvoluted XPS spectra of N 1s, O 1s, and Hg 4f for the N-CQDs@GACTS film before and after Hg\(^{2+}\) sensing. Three distinct peaks at 398.9,
Figure 7. Optimized geometric structures of (a) free N-CQDs@GACTS; (b) [N-CQDs@GACTS]⊃[Hg²⁺] 1:1 complex in the center N-CQDs moiety; (c) [N-CQDs@GACTS]⊃[Hg²⁺] 1:1 complex in one side amide moiety; (d) [N-CQDs@GACTS]⊃[Hg²⁺] 1:2 complex in two amide moieties; (e) [N-CQDs@GACTS]⊃[Hg²⁺] 1:2 complex in center N-CQDs and one side amide moieties; and (f) [N-CQDs@GACTS]⊃[Hg²⁺] 1:3 complex in center N-CQDs and two side amide moieties. (Color code: carbon = gray; nitrogen = blue; oxygen = red; Hg²⁺ = green. All hydrogens are omitted for clarity).

Table 3. DFT Calculated Interaction Energies (ΔIE, kJ/mol) of the N-CQDs@GACTS Hydrogel Unit with Metal Ions (M²⁺ = Hg²⁺, Cd²⁺, and Pb²⁺) in the Water Solvent System

| complex | 1:1² | 1:1³ | 1:2 | 1:2⁴ | 1:3⁵ |
|---------|------|------|-----|-----|-----|
| [N-CQDs@GACTS]⊃[Hg²⁺] | −1581.69 | −1412.77 | −4125.29 | −4197.10 | −6931.57 |
| [N-CQDs@GACTS]⊃[Cd²⁺] | −1429.67 | −1371.50 | −3946.22 | −3969.25 | −6456.45 |
| [N-CQDs@GACTS]⊃[Pb²⁺] | −1210.34 | −1003.09 | −2476.13 | −2560.10 | −5208.83 |

²Note: (a) [N-CQDs@GACTS]⊃[M²⁺] 1:1 complex in the center N-CQDs moiety; (b) [N-CQDs@GACTS]⊃[M²⁺] 1:1 complex in one side amide moiety; (c) [N-CQDs@GACTS]⊃[M²⁺] 1:2 complex in two amide moieties; (d) [N-CQDs@GACTS]⊃[M²⁺] 1:2 complex in center N-CQDs and one side amide moieties; and (e) [N-CQDs@GACTS]⊃[M²⁺] 1:3 complex in center N-CQDs and two side amide moieties. (The optimized geometric structures with bond lengths (Å) are shown in Figure 7 and Figures S2 and S3)
Two types of 1:2 \([\text{N-CQDs@GACTS}] \square [M^{2+}]\) complexation modes can be envisioned. In one scenario, metal ion complexes are formed at two amide moieties of the N-CQDs@GACTS side chains, as shown in Figure 7d (Figures S2d and S3d). The second scenario can be seen where the metal ion complexes are formed in the center of the cavity between the two tetrameric units and at one side amide moiety of the N-CQDs@GACTS hydrogel units, respectively (Figure 7e and Figures S2e and S3e). The DFT calculation results suggest that the \([\text{N-CQDs@GACTS}] \square [M^{2+}]\) 1:2 complex proposed in the second scenario (Figure 7e and Figures S2e and S3e) is energetically more favored than the first scenario. The interaction energies for \([\text{N-CQD@GACTS}] \square [M^{2+}]\) 1:3 complexes with three heavy metal ions (Hg\(^{2+}\), Cd\(^{2+}\), and Pb\(^{2+}\)) are \(-6931.57\), \(-6456.45\), and \(-5208.83\) kJ mol\(^{-1}\). The selected parameters of the bond lengths for \([\text{N-CQD@GACTS}] \square [M^{2+}]\) 1:3 complexes are also shown in Figure 7f and Figures S2f and S3f. The DFT calculation results indicate that the interaction energy of a single N-CQDs@GACTS hydrogel unit with Hg\(^{2+}\) is the strongest, which helps to explain the excellent sensing selectivity of the as-prepared hydrogel to Hg\(^{2+}\).

**CONCLUSIONS**

A novel N-CQDs@GACTS hydrogel film was prepared in this study and was used as an optical sensor to detect traces of heavy metal ions from water solution. FTIR and XPS analyses of the N-CQDs@GACTS hydrogel film indicate that N-CQDs were embedded into the cross-linked hydrogel matrix by weak hydrogen bonds and/or electrostatic attraction. The resultant composite hydrogel exhibited high sensitivity, superior selectivity, rapid response, and a linear detection range of Hg\(^{2+}\) in aqueous media. The porous structure of the N-CQDs@GACTS hydrogel is believed to render a fast mass transfer of Hg\(^{2+}\) onto the hydrogel surface. The high oxidizing ability and strong chelating power of Hg\(^{2+}\) make it readily bind to polar functional groups on the N-CQDs@GACTS hydrogel, resulting in the formation of new complexes through coordination bonds. The DFT computational analyses strongly support our experimentally obtained results that the interaction between the N-CQDs@GACTS hydrogel and Hg\(^{2+}\) is the strongest. The high-resolution XPS results of N 1s and O 1s support the formation of complexes between Hg\(^{2+}\) ions and the N-CQDs@GACTS hydrogel film, which provokes an effective electron transfer for the fluorescence quenching of the N-CQDs@GACTS hydrogel.

**MATERIALS AND METHODS**

**Materials.** ACS grade citric acid anhydrous, ethylenediamine (99%), chitosan (85% degree of deacetylation, MW, 1526 Da), ACS grade glacial acetic acid, glutaraldehyde (25% aqueous solution), ACS grade nitrate salts (Cd(NO\(_3\))\(_2\), Cd\(_{2}\)(NO\(_3\))\(_4\), Hg(NO\(_3\))\(_2\), H\(_2\)O, Pb(NO\(_3\))\(_2\), Zn(NO\(_3\))\(_2\), H\(_2\)O, Cu(NO\(_3\))\(_2\), 3H\(_2\)O, Fe(NO\(_3\))\(_3\), H\(_2\)O, Ca(NO\(_3\))\(_2\), 4H\(_2\)O, and NaNO\(_3\)) were purchased from Fisher Scientific (Canada). These chemicals were used as received without further purification. If not particularly indicated, all solutions were prepared with deionized (DI) water from a Milli-Q water purification system (Millipore Corporation).

**Hydrothermal Method for Fluorescent N-CQDs.** To prepare the N-CQDs, 3.842 g (0.02 mol) of citric acid was mixed with 1.202 g (0.02 mol) of ethylenediamine in a 100 mL Teflon-lined autoclave chamber containing 40.0 mL of DI water and heated in an oven at 180 °C for 4 h. After heating, the autoclave was allowed to naturally cool down to room temperature. The obtained dark-brownish solution was centrifuged at 4000 rpm for 0.5 h and filtered through 0.22 μm membrane filters. Filtrates of the brownish solution were then kept in a dialysis bag in ultrapure water for 8.0 h, and finally the solid N-CQDs were collected after freeze-drying. Solid N-CQDs were redispersed in ethanol/water (1:1 v/v) solution and stored at 4 °C for further use.

**Preparation of the N-CQDs@GACTS Hydrogel Film.** In a typical procedure, a chitosan (CTS) solution of 1.5% (w/v) was prepared by dissolving 0.30 g of chitosan in 20.0 mL of glacial acetic acid (1% v/v) solution and magnetically stirred at room temperature for 30 min. Cross-linking of the chitosan hydrogel was carried out by dropwise addition of 35.0 μL of 0.25 M glutaraldehyde (GA) to the chitosan solution (for 10% degree of cross-linking) under constant shaking at 50 °C for 2 h. After that, 2.0 mL of the N-CQDs solution (20.0 g L\(^{-1}\) ethanol/water solution) was added dropwise to the GACTS hydrogel to obtain the N-CQDs@GACTS hydrogel. After vigorous stirring for 15 min and ultrasonication for another 15 min, the N-CQDs@GACTS hydrogel was decanted on a PVC plate to form thin films, which were then dried overnight at room temperature.

The proposed synthetic route of the N-CQDs@GACTS hydrogel film can be found from Scheme S1 in the Supporting Information.

**Selective Sensing of Hg\(^{2+}\) Ions.** The selectivity of the N-CQDs@GACTS hydrogel was explored under identical conditions with eight metal ions, including three toxic heavy metals (Cd\(^{2+}\), Hg\(^{2+}\), and Pb\(^{2+}\)) and some borderline and hard acids (e.g., Zn\(^{2+}\), Cu\(^{2+}\), Fe\(^{3+}\), Ca\(^{2+}\), and Na\(^{+}\)) typically existing in water. Solutions with two concentration levels (100.0 and 1.0 × 10\(^{-5}\) nM) were prepared for each metal ion. The as-prepared test solutions (2.5 mL) were allowed to interact with the N-CQDs@GACTS hydrogel (0.5 mL) for 1 min at room temperature, and then, the fluorescence emission intensities (\(λ_{em} = 450\) nm) of the hydrogel were measured in the presence (I) and in the absence of (I\(_0\)) interference at an excitation wavelength of 350 nm (\(λ_{ex} = 350\) nm). To test the repeatability of the data, the assay was triplicated for each sample solution.

The sensitivity of the N-CQDs@GACTS hydrogel to Hg\(^{2+}\) was examined using the same procedure by adding a 2.5 mL solution with different concentrations of Hg\(^{2+}\) ions (0–1.0 × 10\(^{-5}\) nM) to a 0.5 mL N-CQDs@GACTS hydrogel. The fluorescence spectra at \(λ_{ex} = 350\) nm were recorded, and all experiments were performed at room temperature.

To investigate the pH effect on the fluorescence intensity of the N-CQDs@GACTS hydrogel and its fluorescence sensing of Hg\(^{2+}\), the N-CQDs@GACTS hydrogel solutions with different pH levels ranging from 2.0 to 9.0 were prepared. The fluorescence intensity was measured in the absence and presence of Hg\(^{2+}\) ions by adding 7.00 μL of Hg\(^{2+}\) solution at 1.0 × 10\(^{-5}\) nM into the hydrogel solution.

**Characterization.** The N-CQDs@GACTS hydrogel film was characterized by various techniques. The Fourier transform infrared (FTIR) spectra of the hydrogel films were measured by a Bruker Tensor II FTIR spectrometer (Bruker, Germany) over a range from 400 to 4000 cm\(^{-1}\). X-ray photoelectron spectroscopy (XPS) measurements were performed using an XPS-Phi 5000 VPH (ULPAC-PHI Inc., Japan) with peak positions being recalibrated by shifting the C
Ds peak to 284.8 eV. A PDI-QuantaMaster spectrophotometer (Photon Technology International Inc., USA) was used to record the excitation and emission spectra of the N-CQDs@GACTS hydrogel before and after metal detection.

**DFT Calculation.** To explore the underlying selective-sensing mechanism of the N-CQDs@GACTS hydrogel, a density functional theory (DFT) study was carried out to investigate the interaction between the hydrogel and metal ions. Compared with other borderline and hard acids (such as Zn$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Ca$^{2+}$, and Na$^+$), Hg$^{2+}$, Cd$^{2+}$, and Pb$^{2+}$ have strong binding affinity to chitosan.$^{31,32}$ Therefore, DFT calculations were carried out in the water solvent system on a model for interactions between a single N-CQDs@GACTS hydrogel unit and three toxic metal ions (Hg$^{2+}$, Cd$^{2+}$, and Pb$^{2+}$). The model structure was composed of two tetrameric N-CQDs units (corresponding to a tetramer form of chitosan) cross-linked with two glutaraldehyde units. All of the computational calculations were carried out with Gaussian 09,$^{33}$ in which geometries of all the structures reported herein were fully optimized in the water solvent system at the B3LYP/GenECIP level of theory. The LanL2DZ$^{34}$ basis set was used for the metals, and the 6-31G(d) basis set was used for all other atoms. The interaction energy between a single N-CQDs@GACTS hydrogel unit and metal ions (M$^{2+}$ = Hg$^{2+}$, Cd$^{2+}$, and Pb$^{2+}$) was calculated by eq 3:

$$
\Delta E = E_{[N-CQDs@GAC]} \Delta E_{[M^{2+}]} + E_{[N-CQDs@GAC]}^{[M^{2+}]},
$$

where $E_{[N-CQDs@GAC]}$ represents the optimized energy of the N-CQDs@GACTS hydrogel complex with a certain metal ion; $E_{[N-CQDs@GAC]}^{[M^{2+}]}$ are the optimized energies of the N-CQDs@GACTS hydrogel unit and the metal ion, respectively.

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