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Full Length Article

Cysteamine-modified diamond nanoparticles applied in cellular imaging and Hg^{2+} ions detection

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ABSTRACT

Luminescent cysteamine-modified nanodiamond particles (**ND-Cys**) have been reported in surface-mediated energy transfer, cell imaging, and metal-ion recognition studies. In normalized photoluminescence (PL) spectral studies, the emission maxima of **ND-Cys** (at 50 µg/mL in water) was fixed at 438 nm at 350 nm excitation, producing a blue emission with a quantum yield (Φ) of 0.13. In the dispersion range of 0–800 µg/mL, **ND-Cys** exhibited agglomeration-induced energy transfer via red shifting of the PL peak from 438 nm to 451 nm. Analogous to PL studies, Raman interrogations also established the agglomeration and fixed the saturation limit to 500 µg/mL in water. The low toxicity and biocompatibility of **ND-Cys** were demonstrated using methyl thiazolyl tetrazolium assay and time-dependent HeLa cell imaging. Subsequently, the Hg^{2+} selectivity by **ND-Cys** was revealed by an intense fluorescence peak shift from 440 nm to 463 nm. Fluorescence studies indicated that the detection limit of Hg^{2+} ions approximated 153 nM. Fourier-transform infrared and X-ray photoelectron spectroscopy (XPS) analyses supported the binding between free thiol (−SH) and amide (−C═O and −NH) groups of **ND-Cys** to Hg^{2+}. The Hg^{2+}-induced agglomeration and surface graphitization were successfully confirmed by X-ray powder diffraction, XPS, Raman, scanning electron microscopy, transmission electron microscopy, dynamic light scattering, and zeta potential analyses. Effective detection of Hg^{2+} ions by **ND-Cys** was validated through HeLa cell imaging at shortened time intervals.

1. Introduction

Nanodiamond (ND)-based research has been attracting the attention of the modern scientific community owing to its wide benefits in diverse fields [1–3]. ND materials have been utilized effectively in semiconductors, catalysis, cell tracking or imaging, drug delivery, and chemotherapeutic and sensory studies [4–9]. In this regard, fluorescent diamond nanoparticle (FND)-based cell tracking or imaging investigations bear importance [10]. Meanwhile, surface-tuned energy transfer and sensory inventions of FNDs are applied in opto-electronic devices [11,12]. Such FNDs can be developed through (1) nitrogen vacancy generation by ionic beam irradiation on synthetic ND, (2) functionalization of ND surfaces by a suitable organic fluorophore, and (3) ND surface passivation by a polymer derivative [13].

Among these methods, the surface modification of ND particles by attaching an organic fluorophore shows potential given the resultant luminescence, photostability, and applicability [14–20]. Such FNDs have been significantly utilized in cellular imaging or tracking research [21]. However, FND-based fluorescent sensors are still required for metal ion detection. To support FND-based metal ion sensors, Berman et al. reported the metal-induced graphitization of diamond particles [22]. In their study, metal ion detection was enabled by rapid tuning of the sp^2/sp^3 ratio of FNDs, thereby boosting the development of surface-modified FNDs for metal ion detection.

Similar to metal ion-induced surface graphitization, the agglomerated surface-induced energy transfer of FNDs has been thoroughly illustrated by various reports [23–25]. The latter strategy may serve as a mechanistic approach in metal ion detection studies. Among metal ions, mercury (Hg^{2+}) ions are widespread in air, water, and soil and can result in massive and harmful environmental impacts [26–29]. Mercury accumulation in the human body may cause a range of cognitive and motor disorders and the Minamata disease and damage the central nervous system [30–36]. Therefore, research groups have focused on developing simple yet effective fluorescent mercury sensors by using

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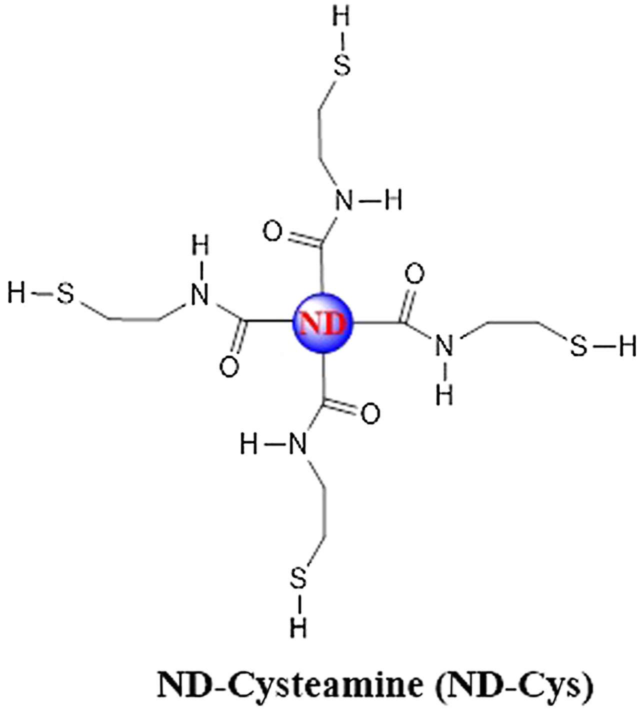

Fig. 1. Structure of ND-Cysteamine (**ND-Cys**).

organic fluorophores or inorganic nanomaterials, such as plasmonic nanoparticles and luminescent metal nanoclusters [37–42]. However, a FND-based fluorescent sensor for mercury ion detection has not been reported. Hence, we headed toward this research direction.

Numerous investigations centered on mercury sensors developed using fluorophores consisting of free thiol or sulfur and amide functionalities [43–46]. We used cysteamine (Cys)-functionalized diamond nanoparticles (**ND-Cys**; Fig. 1) as a probe; we have previously utilized this material in synthesizing diamond nanowires [47]. **ND-Cys** contain Cys as an organic unit on the surface of diamond nanoparticles associated via amide linkages. **ND-Cys** also possess a free thiol (—SH) group and may hence show a high tendency to detect Hg^{2+} ions.

For the first time, we attributed our findings to the biocompatibility and photoluminescence (PL)-based applications of **ND-Cys**. **ND-Cys** displayed a blue emission with a quantum yield (Φ) of 0.13 and manifested agglomerated surface-induced energy transfer via fluorescence changes. The low toxicity of **ND-Cys** has also been demonstrated through cell viability and imaging investigations. More importantly, **ND-Cys** exhibited selectivity toward Hg^{2+} ions over other metal ions; during Hg^{2+} assays, the PL peak of **ND-Cys** red-shifted through surface graphitization [48–50] and agglomeration-tuned intramolecular charge transfer [23–25]. Subsequently, the effectiveness of the **ND-Cys**-mediated Hg^{2+} assay was supported by HeLa cell imaging studies.

2. Materials and methods

2.1. General information

ND-Cys were synthesized and characterized in accordance with our previous report [47]. Fluorescence spectra were obtained using a HITACHI F-7000 fluorescence spectrophotometer. Scanning electron microscopy (SEM) studies were carried out using JEOL-JSM-6700. Transmission electron microscopy (TEM) studies were conducted on JEOL-JEM-2100. The size distributions and zeta potentials (ζ) were obtained using a dynamic light scattering (DLS) BECKMAN COULTER Delsa™ Nano C particle analyzer. Fourier-transform infrared (FTIR) investigations were performed using a Perkin Elmer-100 FTIR SPECTRUM ONE spectrometer. Raman interrogations were implemented using HOROBA and a DPSS 488 nm laser on a Lab RAM HR instrumental set up. X-ray photoelectron spectroscopy (XPS) was performed using Microlab-350 (Thermo Electron Corporation). Fluorescence microscopic images were obtained through a Leica TCS SP5 X AOBS confocal fluorescence microscope (Germany) using a 63 × oil-immersion objective lens.

2.2. Hg^{2+} detection studies

To avoid controversy in **ND-Cys**-based pH-induced surface graphitization during diamond nanowires synthesis [47], we opted not to explore the pH effect on Hg^{2+} determination. Hence, all titrations were performed in deionized (DI) water (at neutral pH) only. Initially, the **ND-Cys** probes were dispersed in water. Metal ions (Na^+, K^+, Ni^{2+}, Fe^{3+}, Cd^{2+}, Ca^{2+}, Ga^{3+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Pd^{2+}, Mg^{2+}, Ba^{2+}, Au^{3+}, Y^{3+}, and Al^{3+}) were dissolved in water medium at 1×10^{-2} M concentration of their respective chloro- and perchlorate compounds. Similarly, Ag^+, Co^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, and Hg^{2+} metal cations were dissolved in water medium at 1×10^{-2} M concentration of their respective acetate salts. Similarly, Cr^{6+} was prepared from potassium dichromate salt. All fluorescence titrations were performed by excitation at 350 nm, and data were collected between 400 and 600 nm. Next, for single analyte studies, PL measurements were obtained by mixing 100 μg of **ND-Cys** with 100 μM of the respective metal ions in water. Similarly, during dual-analyte studies, 100 μg of **ND-Cys** was mixed with 100 μM of Hg^{2+} and 100 μM of the respective metal ions in water. Anthracene in ethanol ($\Phi = 0.27$) was used as reference standard in Φ measurements.

2.3. DLS and SEM

For the ζ and DLS studies, defined concentrations of **ND-Cys** were dispersed in water and utilized in data collection. Conversely, the SEM images of 10 μg/mL **ND-Cys** with metal ions (10 μM) were obtained in water. During SEM measurements, the samples were drop- casted on Si wafer and dried at 50 °C for 15 min prior to data collection.

2.4. TEM studies

TEM analysis of **ND-Cys** at the agglomerated state was conducted at 50 and 950 ng/mL in a copper–carbon grid. Similarly, **ND-Cys** (100 ng/mL) with Hg^{2+} ions (100 nM) were considered.

2.5. FTIR, Raman and XPS spectra [49,51]

For FTIR, Raman, and XPS analyses, the samples (100 μg/mL) were drop-casted on Si wafers and then annealed at 60 °C for 30 min. We used a clean Si wafer as a background reference for both spectroscopic analyses. During Raman interrogations, each measured spectrum was obtained from 20 collected data, wherein four different locations of each sample were considered from five samples. The reproducibility of the presented data is higher than 81%.

2.6. XRD of **ND-Cys** and **ND-Cys** with metal ions

The XRDs of **ND-Cys** in the presence and absence of metal ions were directly obtained through powder XRD analysis. **ND-Cys** were stirred with the metal ions in water for 24 h, centrifuged and washed several times with DI water to remove the excess metal ions adsorbed on the surface of **ND-Cys**, and vacuum-dried in an oven. The dried powder was subjected to XRD analysis. The reproducibility of the presented data is higher than 76%.

2.7. Cell culture of **ND-Cys** in HeLa cell line

The HeLa cell line was provided by the Food Industry Research and