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Photoluminescent Detection of Nitrite with Carbon Nanodots Prepared by Microwave-assisted Synthesis

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A photoluminescent detection method for nitrite with high selectivity and sensitivity using carbon nanodots (CNDs) is demonstrated. The selectivity of nitrite is accomplished by a highly specific diazotization reaction between nitrite and *p*-phenylenediamine (*p*-PDA). In the presence of nitrite, *p*-PDA easily reacts to form the diazonium cation in the acidic aqueous solution. By alkalization of the reaction mixture, diazonium cation of *p*-PDA was converted to an aryl radical to form aggregated CNDs, which causes the change in the photoluminescent intensity of CNDs. In the present method, nitrite can be selectively detected down to 1 μM over several anions, such as nitrate, perchlorate, sulfate, fluoride, chloride, and bromide at mM levels.

Keywords Carbon nanodots, photoluminescence, nitrite, *p*-phenylenediamine

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Introduction

Highly efficient and stable photoluminescent probes are of great importance in analytical and biomedical research. Studies have been focused on the exploration of new efficient photoluminescent materials, such as semiconductor quantum dots,[1] silicon-dye hybrid nanoparticles,[2] and metal nanoclusters.[3] Photoluminescent carbon nanodots (CNDs) prepared *via* several synthetic methods are of particular interest because of their higher photoactivity, lower toxicity, and less-expensive cost compared with heavy-metal contained quantum dots.[4] An increasing number of applications based on CNDs have been carried out, such as cell imaging,[5] dye degradation,[6] pH determination,[7] chemiluminescence,[8] and immunoassay.[9] These CND applications make the most of several favorable properties of CNDs such as size- and excitation wavelength-dependent luminescence emission, photostability, and biocompatibility.[10] Analytical applications of CNDs have been also reported for the detection of metal ions[11] and phosphate,[12] utilizing CND-metal ion affinity based on the carboxylic functionality of the CNDs surface. In recent years, aryl radical reactions were applied to modify nanocarbons, such as carbon nanotubes[13] and graphene,[14] mainly for the effective dispersion in solvents by introducing benzene-sulfonic groups.

As a new detection system for nitrite based on the photoluminescence (PL) intensity change of CND, we report here the aryl radical-induced aggregation of CNDs, in which aryl radicals generated from diazotized *p*-phenylenediamine (*p*-PDA) in the presence of nitrite is regarded as a molecular-glue for the aggregation of CNDs. From the changes in PL intensity of CND in the presence of nitrite and *p*-PDA, it is concluded that the aryl radicals generated from *p*-PDA specifically induce the aggregation of CNDs. In this system, the degree of PL intensity change of CND was found to be proportional to the concentration of nitrite, resulting in the change in the PL intensity of CND to allow selective detection of nitrite down to 1 μM over other anions. Such a selective change in the PL spectrum is one of the essential requirements for analytical use of CNDs, but very few CND-based detection systems with selectivity toward target ions have been reported so far. The present system based on the chemical reactivity of CND is clearly different in principle from some CND-based sensors with metal-carboxylic functionality interaction,[11,12] which have been mainly applied to the detection of metal cations.

Experimental

Reagents and apparatus

Glucose (Wako Pure Chemicals, > 98.0%), poly(ethylene glycol) (PEG-200, Aldrich), 1,4-phenylenediamine dihydrochloride (*p*-PDA, Tokyo Chemical Industry, > 98.0%), 1,2-phenylenediamine dihydrochloride (*o*-PDA, Tokyo Chemical Industry, > 98.0%), and *p*-toluidine hydrochloride (Wako Pure Chemicals, > 98.0%) were used as received. Other reagents were of guaranteed-grade, and were used without further purification. All aqueous solutions were prepared using water

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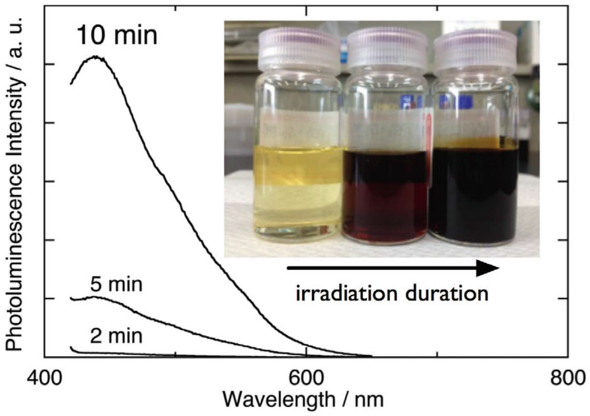


Fig. 1 PL spectra of centrifuged CND solutions prepared by different microwave irradiation time (t to b) 10, 5, and 2 min. λ_{ex} = 360 nm. Inset: Photograph of the stock solution of CNDs.

treated by a Milli-Q system (Millipore Corp., Bedford, MA). UV-vis absorption and photoluminescence spectra were measured by a UV-vis spectrophotometer (JASCO, V-570) and a fluorescence spectrophotometer (Hitachi, F-2500), respectively. The quantum yield of CND was determined using quinine sulfate[15] (Φ = 0.546 in 0.5 M H_2SO_4) as a standard. A pH meter (Horiba, Model F-52) equipped with a combination glass electrode (Horiba, 9611-10D) was calibrated by standard buffer solutions (pH 12.45, 9.18, 6.86, 4.01, and 1.68 at 25°C).

Microwave-assisted synthesis of CND

According to the literature,[16] microwave-assisted synthesis of photoluminescent CND was conducted. Briefly, a 2-gram portion of glucose was dissolved in 3 mL of water and mixed with 10 mL of PEG-200 to form a homogeneous solution. Then the solution was transferred to a PFA pressure vessel (60 mL, Flon Industry) and irradiated in a microwave oven (600 W, Hitachi) for 2 - 10 min. As shown in Fig. 1 inset, the color of crude products changed from colorless to yellow (after 5 min of heating) and finally to dark brown (10 min), which implied the formation of CNDs. After the 10 min of microwave treatment, the crude product was cooled to room temperature and dispersed in water, followed by centrifuging (4000*g*, TOMY Seiko, SRX-201) for 30 min. With a molecular weight cutoff membrane (Amicon Ultra-4, Millipore), CND stock solutions were obtained as equivalent to < 10 kDa. In the PL measurements, as samples were diluted from the crude product by a factor of 10000, PEG concentrations were kept less than 0.01 wt%[17] to avoid the micellar formation. As shown in Fig. S1 (Supporting Information), the liner relationship between the absorbance at the excitation maximum (A_{353}) and the maximum PL intensity (I_{455}) was confirmed up to A_{353} = 0.2. Due to the coexistence of PEG in the synthesis procedure, CND could not be isolated by the centrifuging purification used in this study. Relative concentrations of CND in the measurement solutions were determined by the absorbance at the excitation wavelength, typically A_{353} = 0.1.

Procedure for nitrite detection

PDA was dissolved in 0.1 M HCl at r.t., then an aqueous solution of sodium nitrite was added and left for 20 min. Final concentrations of *p*-PDA and nitrite in the mixture were 10 µM and 1 - 50 µM, respectively. After neutralization by addition of NaOH, CND and CAPS buffer (pH 10.2) solutions were added to the mixture and left for another 40 min. Consequently, PL spectra of the mixture were recorded after 60 min from the addition of nitrite.

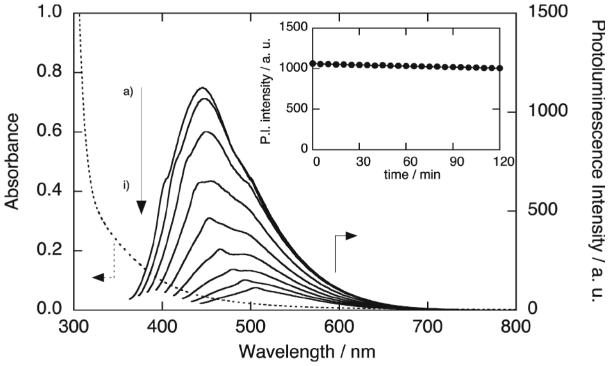


Fig. 2 Absorption (dotted line, left axis) and emission (solid lines, right axis) spectra of CND dispersed in water. λ_{ex} were a) 353 to i) 433 nm with an increment of 10 nm. Inset: Time course of PL intensity under continuous irradiation of excitation light (λ_{ex} = 353 nm) for 2 h.

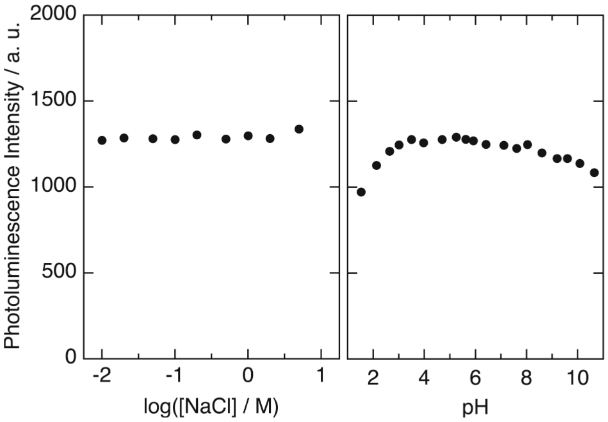


Fig. 3 Left: Plots of PL intensity *versus* NaCl concentrations. [NaCl] = 0.01 – 5 M, λ_{ex} = 353 nm. Right: Plots of PL intensity *versus* pH values. pH = 1 – 11, λ_{ex} = 353 nm.

Results and Discussion

Photoluminescence property of CNDs

Figure 2 presents the absorption and emission spectra of the diluted CNDs. CNDs exhibit an emission peak at 445 nm in the excitation at 353 nm, with a maximal emission wavelength shift of approximately 80 nm when the excitation wavelength increases from 353 to 433 nm. CNDs also showed excellent photostability, as the PL intensity change was less than 3% under continuous irradiation of excitation light for 2 h (Fig. 2 inset). It should be mentioned here that the PEG itself and microwave-treated one are non-emissive in the visible region, confirming the bright photoluminescence to be originating from the synthesized CNDs. The quantum yield of the CND was determined to be 0.007 (λ_{ex} = 353 nm) and the value could be comparable to those of CNDs obtained by other groups,[18,19] As shown in Fig. 2, the PL spectra of the CNDs are generally broad and dependent on excitation wavelengths, which may reflect not only effects from particles of different sizes in the sample but also a distribution of different emissive sites on each CND

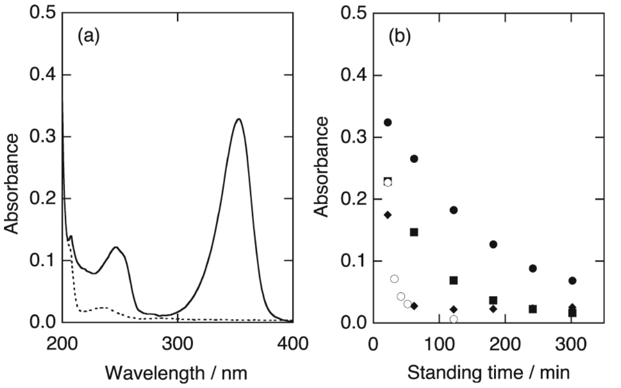


Fig. 4 (a) Absorption spectra of 10 μM *p*-PDA in the absence (dotted line) and presence (solid line) of 20 μM $NaNO_2$ at pH 1.1. (b) Decay curves for absorption maximum (λ = 352 nm) of diazotized *p*-PDA at different pH levels. ● pH 1.1, ■ pH 5.8, ◆ pH 9.3 and ○ pH 10.2. At the time that the reaction mixture was neutralized by addition of NaOH is defined as t = 0 min of this plot.

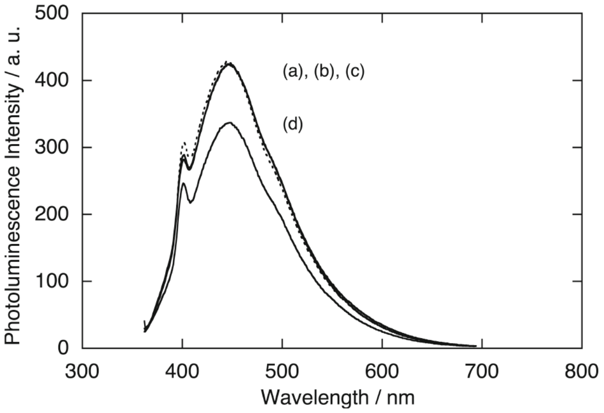


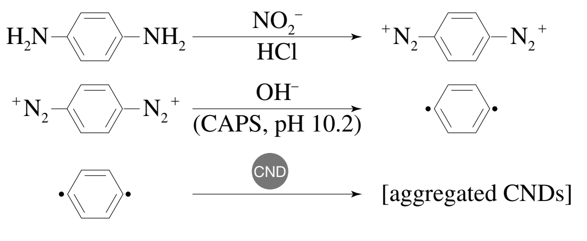
Fig. 5 PL spectra of CNDs after reaction with diazotized anilines generated by $NaNO_2$ (20 μM) at pH 10.2, (a) in the absence of anilines, and presence of (b) *p*-toluidine (20 μM), (c) *o*-PDA (10 μM), and (d) *p*-PDA (10 μM).

particle.[20]

The PL stability of the CNDs was further examined under different conditions (Fig. 3). The PL intensity remained almost constant (± 4%) in aqueous solutions containing NaCl at concentrations of up to 5.0 M. In addition, the PL intensity was rather pH independent in the pH range 3 – 10. The gradual decrease observed at pH 1 – 3 was ascribed to the decrease in the density of deprotonated carboxylic acids functionality on the CND surface.[21] These results suggest the high dispersibility of the CNDs in a wide range of aqueous solutions.

Aryl radical generation from diazotized p-PDA

The detection of nitrite with CNDs was designed as a one-pot procedure with three individual steps; i) diazotization of *p*-PDA by nitrite,[22] ii) generation of aryl radicals from diazotized *p*-PDA by alkalization and iii) formation of aggregated CNDs by the spontaneous reaction of aryl radicals, *i.e.*,



Prior to the addition of CND to the *p*-PDA-nitrite mixture, generation of aryl radicals from diazotized *p*-PDA was a key step for this method. In the absence of nitrite, *p*-PDA exhibited a small absorption at 280 nm at pH 1.1, whereas significant absorption was recognized at 352 nm after the addition of nitrite as shown in Fig. 4. This new absorption is characteristic of the absorption maxima of diazonium ions that appear in the range of 330 – 380 nm.[23] The absorbance at 352 nm increased up to 20 min and then gradually decreased for 5 h (figure not shown), indicating the slow decomposition of the diazotized *p*-PDA under acidic conditions.

Since the absorption band of diazotized *p*-PDA and the excitation maximum of CND overlapped, it is essential to convert diazotized *p*-PDA to aryl radicals completely. We considered two criteria from the complete decrease in absorbance of diazotized *p*-PDA at 352 nm for the precise determination of nitrite by the reaction with CNDs. First, we ensured that the inner-filter effect by diazotized *p*-PDA was minimal. Second, the possibility of charge interaction between diazotized *p*-PDA and CNDs was excluded. As for the reaction between carbon substrates and diazonium compounds in a homogeneous solution, it is well accepted that the reaction proceeds, through reductive radical formation with loss of nitrogen, by spontaneous reactions promoted by chemical reducing agents, heat, or UV irradiation.[24] To facilitate the reductive decomposition of diazotized *p*-PDA, we tested the effect of hypophosphorous acid[25] as a reducing agent. Although the decay time was shortened by the addition of hypophosphorous acid, it took, however, more than an hour to exhibit negligible small absorbance (> 0.02) at 352 nm (figure not shown). On the other hand, the increase in the pH value of the reaction mixture had a significant effect on the decomposition of diazotized *p*-PDA and generation of aryl radicals.[26] Figure 4(b) shows the decay curves of the absorbance at 352 nm at different pH values. By increasing the pH value to 10, the absorbance at 352 nm decreased to a negligible small value within an hour, reflecting the complete decomposition of diazotized *p*-PDA. Therefore, the nitrite detection procedure was adopted as *p*-PDA-nitrite reaction for 20 min in an acidic solution to form diazotized *p*-PDA, followed by the addition of CNDs and alkaline buffer (CAPS, pH 10.2); the mixture was then allowed to stand for another 40 min to decompose diazotized *p*-PDA and generate the aryl radicals.

Reactivity of aryl radicals with CND

Probably due to the coexistence of PEG-200 in the CND solution, reliable results were not obtained by dynamic light scattering and transmission electron microscope measurements to show the direct evidence for reaction between aryl radicals generated from diazotized *p*-PDA and CNDs. Therefore, under the proposed experimental conditions, the reactivity of aryl radicals was evaluated by comparing with other aniline derivatives having different number and position of amines. Figure 5 displays PL spectra of the CND solution containing aryl radicals generated from various aromatic amines, such as *o*-PDA, *p*-toluidine, and *p*-PDA, diazotized by sodium nitrite. The concentrations of anilines were 10 or 20 μM on the basis of the number of amines, which is equivalent to nitrite (20 μM). As shown in Fig. 5, the PL intensity at 445 nm is quenched by

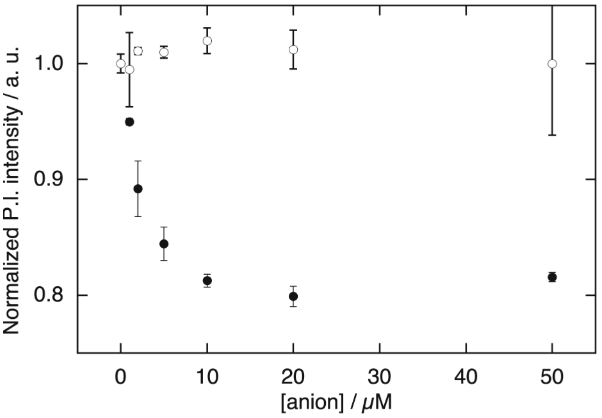


Fig. 6 Changes in the PL intensity of CNDs in the presence of *p*-PDA and (○) NO_3^- or (●) NO_2^-. [PDA] = 10 μM, [anion] = 1 – 20 μM, [buffer] = 20 mM, pH 10.2

as much as 21% for the case of diazotized *p*-PDA. The PL quenching is negligibly small for other aniline derivatives (less than 1% for *o*-PDA and *p*-toluidine). Apparently, the PL intensity of CND is strongly influenced by the number and position of amines in the corresponding aniline derivatives. This result demonstrates that the CND aggregates in solution with the assistance of aryl radicals generated from diazotized *p*-PDA. Because each *p*-PDA molecule has two pairs of amines in para position, *p*-pheylene radicals formed can readily react with separate CNDs and make aggregates effectively. With the formation of aggregated CNDs, the PL of the CNDs is quenched, which is considered as the surface quenching states-induced mechanism.[27]

Detection of nitrite with CND

Figure 6 shows the dependence of the PL intensity on the concentrations of nitrite and nitrate. With an increase in nitrite concentration, the PL intensity of the CNDs decreased after the reaction with diazotized *p*-PDA at pH 10.2. Since the diazotization reaction proceeds quantitatively,[23] PL intensity change is recognized for 1 – 20 μM nitrite (0.1 – 1.0 eq. for 10 μM *p*-PDA). It is noteworthy that the presence of 50 μM nitrite of which the equivalent is 2.5 times larger than *p*-PDA added gave no significant change on the decrease of PL intensity. Concerning interference by nitrate, the CNDs show negligibly small changes in PL intensity for the concentrations ranging from 1 to 50 μM. The effects of other anions on the PL intensity were investigated by adding various sodium salts under the optimum conditions. The results are summarized in Table 1, from which it can be seen that some common anions hardly affect PL intensity within the relative error of 2%. Thus, our proposed approach was found to exhibit high selectivity for nitrite in water.

Conclusions

We have shown the CND to be a novel photoluminescent probe for nitrite based on the specific reaction *via* aryl radicals generated from *p*-PDA diazotized by the nitrite. The combination of CND and aryl radical reaction provides an effective aggregation of CNDs to show PL intensity change, resulting in a selective detection of nitrite. The present system presents evidence of the value of the chemical reactivity of CND itself, in addition to the oxygenated functional groups on the CND surface, for the development of novel CND sensors. Because of the dispersibility of CNDs in high ionic strength and wide pH range in aqueous solutions, this method can be carried out with a one-pot procedure, making it a convenient and cost-effective method for the development of CND based-sensors.

Table 1 Effect of foreign anions on the PL intensity for 20 μM nitrite in the present method (*n* = 3)

Anion	Concentration/μM	Relative error, %
NO_3^-	100	0.7
	1000	0.8
SO_4^{2-}	100	1.6
	1000	0.5
ClO_4^-	100	1.0
	1000	1.0
F^-	100	0.9
	1000	1.0
Cl^-	100	1.4
	1000	0.3
Br^-	100	0.6
	1000	0.7

Acknowledgements

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Supporting Information

The method of quantum yield determination and linear relationship between absorbance at the excitation maximum and PL intensity of CNDs. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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