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Rapid "turn-on" detection of atrazine using highly luminescent N-doped carbon quantum dot

Sasmita Mohapatra*, Mihir K. Bera, Rahul K. Das

Department of Chemistry, National Institute of Technology, Rourkela, Odisha, 769008, India

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ABSTRACT

Nitrogen doped carbon quantum dot based highly selective luminescent probe has been designed for the detection of herbicide atrazine. Nitrogen doped carbon quantum dot with PLQY 28% has been prepared from hydrothermal treatment of orthophenylene diamine. The turn-on fluorescence of the probe on addition of atrazine is attributed to aggregation induced fluorescence enhancement of amine functionalized carbon dot. The hydrogen bonding interaction of surface amine groups of carbon dot probe and atrazine makes the probe selective and ultrasensitive towards atrazine even in presence of other pesticides and metal ions. The limit of detection for atrazine sensing was found to be **3 pM,** which is the lowest among all reported methods for the detection of atrazine. Due to low detection limit the utility of the developed sensor has also been explored in real agricultural samples. Owing to the excellent luminescence property and photostability this fluorescent probe can also be utilised to detect atrazine in bacterial cell by imaging.

1. Introduction

The advancement in the area of pesticides and herbicides for the control of a wide variety of insectivorous and herbaceous pests has been a fundamental contributor to the green evolution [1]. However with the benefit of pesticides there exist some hazardous impacts like the long-term survival of major ecosystems and biodiversity as well as oncological, pulmonary and haematological morbidity in human [2–5]. Atrazine (6-chloro-*N*-ethyl-*N*′-isopropyl-1,3,5-triazine-2,4-diamine) is a conventional triazine herbicide with better efficiency to control broad spectrum of weeds in corn, sugarcane, pineapple, and other culture [6–8]. Due to its low reactivity, water solubility and slow degradation, ATZ is reasonably stable, mobile and persistent in the environment for a longer period [9]. In very small quantities, it may act as a carcinogen of type C and an endocrine disruptor of the hormonal system. Continuous exposure to atrazine can cause immune suppression, reproductive abnormalities, carcinogenicity and hormone disruptions [10]. As per to the World Health Organization (WHO) maximum contaminant level (MCL) of atrazine in drinking water and crop is reported to be in a range of 20–250 ng g^{-1}. Thus it is very much important to build up powerful and efficient methods to determine the residues of atrazine herbicide in different agriculture samples. Various analytical methods such as gas chromatography, HPLC, spectrophotometric, colorimetric methods have been reported for sensing trace amount atrazine in environmental samples [11–14]. However these analytical techniques suffer from severe drawbacks like complicated sampling techniques, time consuming sampling, requirement sophisticated instruments, highly trained technicians and inability to perform onsite detection. To overcome these problems electroanalytical techniques have been reported [15–17]. Most of the electroanalytical techniques involve electrochemical sensors based on molecularly imprinted polymers. Although these methods are highly selective for detection of atrazine, it embraces tedious electrode preparation and delicate sampling techniques. Recently, Wang et al. have published colorimetric detection of atrazine using cysteamine functionalized Au nanoparticles [18]. The presence of atrazine can induce aggregation of Au nanoparticles and shifting of SPR absorption band. Even though this method presents a simple naked eye detection of atrazine, the sensor is not cost-effective for large scale application. Therefore, the prime challenges in fabricating ATZ sensor are (1) high sensitivity and selectivity in detection, (2) consist of stable receptors that associate with analytes selectively, (3) retain high aqueous solubility and reproducibility, (4) cost-effectiveness and (6) most importantly low detection limit of the sensor for reliable analytical performance in real samples.

Fluorescence sensing has been emerging as an efficient detection method because of its high sensitivity, spatiotemporal resolution and versatility [19,20]. Advanced techniques based on

* Corresponding author.
E-mail address: sasmitam@nitrkl.ac.in (S. Mohapatra).

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quantum dot based fluorescence detection may reduce the cost of the sensing process. Recently carbon dots (CDs) have emerged as promising fluorophores in sensing and imaging applications because of its easy preparation, high PLQY, photostability and tunable luminescence property [21]. The unique photoluminescent property of CD has been explored for sensing a wide range of cations and anions like Fe^{2+}, Sn^{2+}, Hg^{2+}, Cu^{2+}, Cl^{-}, I-, F- and so on [22–26]. Application of carbon dot towards fluorescence detection of salt ions, organic/biological molecules, and target gases is well reviewed by Sun et al. [27]. The basic sensing mechanism in case of carbon quantum dots is based on PL quenching of C-dot upon aggregation, electron/energy transfer or fluorescence recovery of the already quenched CD-quencher complexes. Although extensive work has been done on rapid detection of metal ions, anions and biomolecules using luminescent carbon quantum dots, the available documentation on development of fluorescence sensors for the detection of small organic molecule pollutants such as pesticides or herbicides is extremely sparse. For instance simple fluorescence detection techniques for few pesticides like sinapine [28], carbaryl [29], paraxon [30], glyphosate [31] have been reported where the limit of detection can go up to ng/mL, sensing methods for common herbicide like atrazine has not been explored. Further, the above reports demonstrate simple protocols to detect different pesticides in real samples like lake water, fruits and vegetables with low LoD, however the detection of pesticides in living biological cells has yet to be explored. The permissible limit defined for most of the modern pesticides are normally obtained from a human health perspective. Even if the concentrations of modern pesticides are far below the detection limit, it is possible that detrimental effects on ecological system exist. Microbial responses have been commended as an early caution indicator of ecosystem stress, because of the quick response to changes in environmental conditions. So we deemed to explore the utility of our sensing protocol in bacterial cells.

In the presented work, we established a novel fluorescent sensor for atrazine detection. We have used highly fluorescent CD as a fluorophore and surface amine groups as receptors to recognize atrazine. The sensing principle involves hydrogen bonding between atrazine and surface NH_2 carbon dots of at lower pH. Upon addition of atrazine to the system, the fluorescence intensity gradually increases due to formation of hydrogen bonding between carbon dots and atrazine which induces aggregation and enhances PL intensity (Fig. 1). This sensor effectively works in a broad linear concentration range (5pM–7 nM). So it has been successfully applied to detect atrazine in real samples like cucumber, lemon, bottle gourd and sugarcane. The proposed sensing method has also been explored for the detection of atrazine in bacterial cells.

2. Experimental

2.1. Materials

o-Phenylenediamine(OPD), quinalphos, imidacloprid, cypermethrin, chloropyrifos were purchased from Sigma Aldrich. Atrazine was obtained from TCI Chemical, Japan. Cucumber, Lemon, watermelon were bought from local market. Dichloromethane was purified by distillation over phosphorous pentoxide. All other chemicals were used as received without further purification. Milipore water (18.2 MΩ) was used throughout the experiment.

2.2. Method

0.9 gm o-phenylenediamine was taken in a beaker and dissolved properly using 90 mL ethanol. The transparent solution obtained was transferred into poly(tetrafluoroethylene)-lined autoclave. The hydrothermal treatment was carried out at 180 °C for 12 h. The solution was allowed cool down to room temperature naturally Then, the obtained dispersion were filtered with a 0.22 μm filter membrane to remove the larger product, and then dialyzed against deionized water by a dialyzator with molecular weight cut off 1000 Da for 24 h. Finally, an orange coloured aqueous solution containing carbon quantum dots (o-PCD) was obtained. The o-PCD was recovered by lyophilizing the solution.

2.3. Atrazine sensing

In a typical experiment, 20 μL of o-PCD solution (0.1 mg/mL), 3 mL H_2O and 1 mM acetate buffer were mixed and incubated for 5 min. Then 0.2 mL of atrazin solution of different concentration was added and incubated at 37° C for 15 mins. The PL spectrum was recorded (λ_{em} = 500 nm at λ_{ex} = 420 nm). To investigate method selectivity, some common potential interfering pesticides such as quinalphos, cypromethene, imidaclopid and chlorpyrifos and metal ions such as Cu^{2+}, Co^{2+}, Ni^{2+} etc were also tested at similar conditions.

2.4. Characterization

Photoluminescence spectra were collected on a Horiba Quantamaster fluorimeter. Morphological, microstructural analysis and elemental mapping were done using a high resolution transmission electron microscope (FEI Technai G2 30ST) operated at 300 kV. The presence of surface functional group was investigated through FTIR recorded by an IRAffinity-1S, Shimadzu, spectrophotometer. Surface composition of oPCD was verified by XPS using AlKα excitation source in PHI 5000 Versa Prob II (FEI Inc) instrument. Particle size and mean zeta potential at different pH values were measured using Nano ZS 90, Malvern instrument.

2.5. Calculation of atrazine recovery in real samples

Edible vegetables such as cucumber, lemon and bottle gourd were collected from local market. 1 g sample was taken and chopped in to small pieces and dissolved in water and ultrasonicated for 2 mins. After keeping for 5 mins different concentrations of atrazine standard solutions were added into the obtained matrix of apple samples. The supernatant was collected for analysis according to the method in Section 2.3. For recovery experiment known quantities of atrazine finely chopped samples then pretreated and analysed according to the above procedure.

2.6. Atrazine sensing experiment in bacteria

Escherichia coli was grown in LB medium and staphylococcus aureus was grown in NB medium overnight at 37 °C. After overnight growth, a colony from each bacterial stain was collected to measure its optical density and was adjusted to 1.0. The bacterial cells were centrifuged and washed twice with sterilized PBS at 5500 rcf for 10 min, and the cell pellet was resuspended in 20 mL PBS. From the E.coli suspension, three aliquots were prepared containing 5 mL of suspension each. First aliquot was maintained as control, second aliquot was treated with 50 μL of o-PCD (2 mg mL^{-1}) after 3 h of incubation whereas the third aliquot was preliminarily treated with 10 μL of atrazine (7 nM) for 3 h and then 50 μL of o-PCD (2 mg mL^{-1}) was added to it and incubated for 4 h. The three suspensions were kept with respect to standard time so as avoid growth difference in each set. To remove the residual o-PCD and atrazine, the mixture was centrifuged and washed twice to get CD-labelled E.coli. The pellet was then resuspended in PBS buffer. From each set, sample was mounted onto glass slides and subjected to confocal microscope (Leica TCS SP8) for imaging. The fluorescent images were