

FABRICATED METAL-FREE CARBON NITRIDE CHARACTERIZATIONS FOR FLUORESCENCE CHEMICAL SENSOR OF NITRATE IONS

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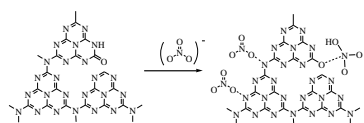
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Graphical abstract



Proposed interactions between the CN and the NO₃⁻ molecules.

Abstract

In this study, a metal-free carbon nitride (CN) was investigated for the first time as a potential fluorescence sensor for detection of nitrate ions (NO₃⁻). The CN was prepared through thermal polymerization of urea precursor at 823 K and characterized by diffuse reflectance ultraviolet-visible (DR UV-Vis), Fourier transform infrared (FTIR), and fluorescence spectroscopies. The DR UV-Vis spectrum confirmed that CN could absorb light up to 450 nm. On the other hand, the FTIR spectrum revealed the presence of graphitic CN single and double bond characters in the 800-1700 cm⁻¹ region. From the fluorescence spectroscopy, three excitation peaks at 278, 310 and 369 nm were observed due to the presence of N=C, C=O and N-C groups, respectively. The fluorescence sensor capability of the CN was then investigated using concentrations of NO₃⁻ in the range of 300-1800 μM. It was confirmed that the intensities of the emission sites were quenched linearly with the concentrations of the NO₃⁻. The CN showed good reproducibility with relative standard deviation (RSD) values were 1.5-7.2%. These results suggested that CN can act as the fluorescence sensor for NO₃⁻.

Keywords: Carbon nitride, fluorescence sensor, nitrate ions, Stern-Volmer plot, quenching rate

Abstrak

Dalam kajian ini, karbon nitrida tanpa logam (CN) telah dikaji buat pertama kali sebagai sensor pendarfluor yang berpotensi untuk mengesan ion nitrat. CN telah disediakan melalui kaedah pempolimeran terma prekursor urea pada 823 K dan telah dicirikan dengan spektroskopi pantulan serakan ultralembayung-sinar nampak (DR UV-Vis), spektroskopi infra merah transformasi Fourier (FTIR) dan spektroskopi pendarfluor. Spektrum DR UV-Vis telah mengesahkan CN dapat menyerap cahaya sehingga 430 nm. Selain itu, spektrum FTIR mendedahkan kehadiran ciri CN grafitik ikatan tunggal dan ikatan kembar pada kawasan 800-1700 cm⁻¹. Daripada spektroskopi pendarfluor, tiga puncak pengujian iaitu 278, 310 dan 369 nm telah dicerap disebabkan kehadiran kumpulan N=C, C=O dan N-C. Seterusnya, keupayaan pengesan pendarfluor CN disiasat menggunakan kepekatan NO₃⁻ yang berbeza dalam julat 300-1800 μM. Telah terbukti keamatan tapak pemancaran telah diilindap secara linear dengan kepekatan NO₃⁻. CN telah menunjukkan kebolehulangan yang baik dengan sisihan piawai relatif (RSD) iaitu 1.5-7.2%. Dapatan ini telah mencadangkan CN boleh bertindak sebagai sensor pendarfluor untuk NO₃⁻.

Kata kunci: Karbon nitride, sensor pendarfluor, ion- nitrat, plot Stern-Volmer, kadar pelindapan

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1.0 INTRODUCTION

Nitrate (NO_3^-) has been classified as an inorganic nitrogenous compound, which can cause disease and become a threat to human health [1]. The NO_3^- can be found naturally in water and food, thus, it can be easily consumed by human. Previous studies showed the healthy range of NO_3^- for adult in urine is between 300 to 1800 μM [2]. High NO_3^- level in human urine represents a significant risk to health, as it is directly responsible for methemoglobinemia or "blue baby syndrome" [3]. The nitrite (NO_2^-) is consequently absorbed by hemoglobin to form methaemoglobin. On the other hand, NO_2^- that is formed by bacteria from NO_3^- also can induce gastric cancer by reacting in stomach with agents of human gastric cancer. Due to the excess of NO_3^- , other symptoms such as abdominal pain and diarrhea have also been observed in human [4].

If the level of NO_3^- in human urine is outside the healthy range, it will create a problem in our body system. Hence, it is a must to develop tools or methods that can be used to determine the presence of NO_3^- in urine. The conventional method to detect NO_3^- in urine analysis is based on a spectrophotometry technique using Griess reagent. Griess reaction is specific for determination of NO_2^- quantification, but it also can be used for determination of total NO_3^- and NO_2^- in a sample. Therefore, when using the Griess reagent, the quantification of NO_3^- has to be determined separately [2]. Unfortunately, the simplest spectrophotometric Griess reaction has numerous meddling factors since they are mostly inaccurate. Besides, the Griess reagent is also difficult to be reused due to the formation of complex compound during reaction with the analyte [5]. Therefore, it is highly important to develop a reusable and sensitive sensor to detect the presence of NO_3^- in human urine.

Many new materials have been developed to overcome these problems. In this study, metal-free carbon nitride (CN) is proposed for the first time as the fluorescence sensor for NO_3^- . The CN is a well-known stable allotrope of graphitic compound under ambient conditions. It is constructed from tri-*s*-triazine units as a building block in the structure, which make up of carbon and nitrogen atoms [6-11]. Recently, the CN has attracted a great deal of scientific interest because theoretical investigations revealed its excellent biocompatibility [6-7], high photoluminescence (PL) intensity [7-9], and good photostability [12-13]. All of these unique characteristics make the CN an ideal platform for photocatalysis [12-14], bioimaging [15], drug delivery [16], and sensing [6-11]. Lee *et al.* first reported cubic mesoporous-ordered graphitic CN as a selective optical sensor for the detection of trace amount of metal ions, such as copper ions [6]. Moreover, it also has been reported to give high sensitive detection to cyanide with low detection limit of 80 nM, not only in aqueous solution but also in human blood serum [7].

The CN also showed good interactions with the N-nitrosopyrrolidine [8]. It was expected that the CN can also interact with other nitrogen-containing compounds. In this study, the CN was used to detect NO_3^- via fluorescence quenching. The NO_3^- ions do not have the fluorescence ability. Therefore, if there are interactions between the emission sites of the CN and the NO_3^- , the fluorescence intensity of the CN will be quenched.

2.0 EXPERIMENTAL

The materials used in this study were urea, sodium nitrate and distilled water. The chemicals were purchased and used without further treatments and purification.

2.1 Procedure

2.2.1 Preparation of CN

A metal-free CN was synthesized by thermal polymerization of urea in a close system according to a reported procedure [14]. In a typical synthesis, urea (25 g, Sigma Aldrich, $\geq 99\%$) as a precursor was placed in an alumina crucible with a cover and then heated at rate of 2.2 K min^{-1} to reach a temperature of 823 K. It was tempered at this temperature for 4 h to give a yellow powder.

2.2.2 Characterizations of CN

The diffuse reflectance spectrum was recorded at room temperature on a Shimadzu UV-Vis spectrophotometry (UV-2600), in which barium sulfate (BaSO_4) was used as the reference. The Fourier Transform Infrared (FTIR) spectrum of CN was recorded using a Nicolet iS50 spectroscopy by mixing the CN with potassium bromide (KBr) as a pellet. The excitation and emission spectra of CN were measured at room temperature by using a fluorescence spectroscopy (JASCO, FP-8500). The spectra were monitored at wavelength of 453 nm for excitation spectra, and 278, 310 and 369 nm for emission spectra measurements.

2.2.3 Quenching Tests

In order to study the interaction between emission sites of CN and the NO_3^- , the quenching tests of CN were carried out at room temperature using a fluorescence spectroscopy. The NO_3^- solution with various concentrations in the range of 300 to 1800 μM was used. The CN (0.2 g) was placed in the solid sample holder and the certain concentration of NO_3^- solution (10 μL) was introduced to the CN. The emission spectra were then measured at excitation wavelengths of 278, 310, and 369 nm.

2.2.4 Reproducibility and Stability Tests

The reproducibility of the CN as the fluorescence sensor was investigated at four repetitions on four batches of the CN. The standard deviations (SD) and the relative standard deviations (RSD) were calculated for each concentration and the limit of detections (LOD) were then determined from the SD and the slope.

The stability of the CN was also evaluated by reusing the CN as the fluorescence sensor for the detection of the NO_3^- . Prior to reusing the CN, the used CN was stirred and washed in distilled water for several times, followed by drying at 353 K for 12 h. The dried sample was then evaluated again for the quenching tests.

3.0 RESULTS AND DISCUSSION

In this study, the CN was synthesized from urea as precursor via thermal polymerization method. The optical property of the CN was confirmed from DR UV-Vis spectroscopy, and the chemical structure was determined from FTIR spectroscopy. In this research, the CN was used as a potential fluorescence sensor for determination of the NO_3^- which concentrations in the range of 300–1800 μM .

Figure 1 illustrates the measured absorption spectrum of the CN by using BaSO_4 as the reference at room temperature. From the figure, it was clear that the CN showed absorption up to 450 nm, which was in good agreement with its yellow color appearance [6–8, 10, 12]. The CN exhibited three absorption peaks at 296, 337 and 396 nm. On the other hand, it was reported that the mesoporous CN prepared by cyanamide only exhibited two peaks at 285 and 380 nm [8]. The peaks at 285 and 380 nm were originated from π to π^* ($\pi \rightarrow \pi^*$) electronic transition in the aromatic 1,3,5-triazine of CN (C=N group) and the excitation of n to π^* ($n \rightarrow \pi^*$), involving lone pair of electrons on the nitrogen atoms (terminal N-C). Hence, the peaks at 296 and 396 nm could be also assigned to C=N and terminal C-N groups, respectively. Furthermore, it was reported that the use of oxygen-containing precursor such as urea caused the incomplete pyrolysis process to form the CN due to the fact that oxygen was difficult to be removed from the CN [10]. This result suggested that the carbonyl group (C=O) group might exist on the CN prepared by urea. Therefore, the peak at 337 nm observed in this study could be corresponded to π to π^* ($\pi \rightarrow \pi^*$) electronic transition of C=O group.

The FTIR spectrum of CN that was mixed with KBr as a pellet was measured at room temperature and shown in Figure 2. The adsorption peaks at 809 cm^{-1} was considered to be the out-of-plane skeletal bending modes of 1,3,5-triazine. The adsorption bands in the range of 1200–1700 cm^{-1} were assigned to the typical stretching modes of CN heterocyclic. The peaks at 1240, 1316, 1410, and 1459 cm^{-1} were assigned to aromatic C-N stretching vibration modes.

The 1545 and 1575 cm^{-1} peaks were attributed to N-H bending vibration mode, while the peak at 1640 cm^{-1} was ascribed to C=O stretching vibration modes. The broad bands in the 3000–3500 cm^{-1} region could be attributed to the stretching vibrations of O-H and N-H components, indicating the existence of uncondensed amino groups and absorbed H_2O molecules in CN [17]. These observed peaks were in good agreement with other reported literatures on the CN prepared by urea [10,13,18].

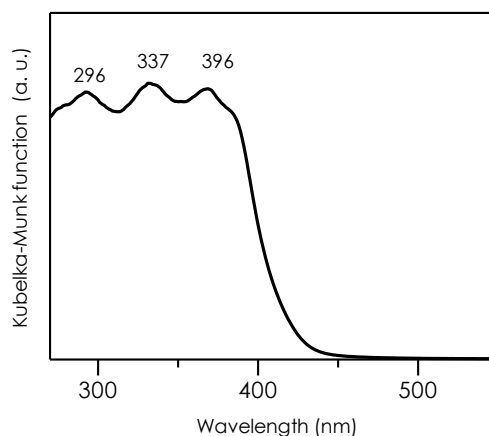


Figure 1 DR UV-Vis spectrum of the CN

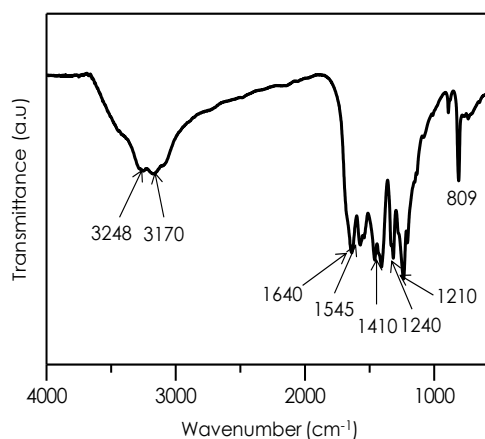


Figure 2 FTIR spectrum of the CN

As for the fluorescence properties, the CN was confirmed to possess three maximum excitations at 278, 310 and 369 nm as shown in Figure 3. This excitation spectrum was in good agreement with the DR UV-Vis spectrum obtained in Figure 1. Analogy to the DR UV-Vis spectrum, the adsorption peak at 278 nm would be originated from the N=C groups ($\pi \rightarrow \pi^*$) in the conjugated aromatic 1,3,5-triazine ring of CN [8]. On the other hand, the peak at 310 nm would be originated from the C=O groups ($\pi \rightarrow \pi^*$) [10], while the peak at 369 nm would be due to the terminal N-C groups ($n \rightarrow \pi^*$) [8] of the CN. From Figure 3, only one emission peak was found at 453 nm when CN was excited at wavelengths of 278, 310 or 369 nm. The emission intensity was in the similar order to the

order of excitation intensity, suggesting that the emission was originated from the respective excitation site. The different peak intensities implied that the emission sites might be different in numbers and/or fluorescence capabilities.

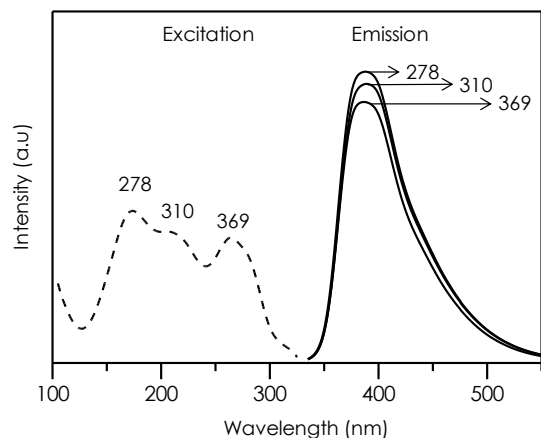


Figure 3 The excitation and emission spectra of the CN.

In order to evaluate the sensitivity of the CN sample toward NO_3^- molecules, the quenching experiments were carried out at room temperature by using fluorescence spectroscopy. The quenching tests were conducted by monitoring the changes of the emission intensity in the absence and presence of various concentrations of NO_3^- in the range of 300–800 μM . The emission spectra were monitored at three different excitation wavelengths, which were 278, 310 and 369 nm. As displayed in Figure 4, the intensities of the emission spectra of the CN at each excitation wavelength were found to decrease with

the addition of NO_3^- . As mentioned above, the NO_3^- ions are not fluorescence molecules. The reduced intensities suggested that all the emission sites excited at these three excitation wavelengths interacted with NO_3^- molecule and the interactions led to the decrease in the emission intensity. The emission intensity of the CN was found to decrease as the concentration of the NO_3^- increased up to 1800 μM . Further increase in the concentration of NO_3^- did not cause further decrease in the emission intensity. This saturation condition was observed since not all of the emission sites of the CN could interact with the NO_3^- molecules.

In order to clarify the favorable emission sites to interact with the NO_3^- molecules, quenching efficiency at each excitation wavelength was determined by a Stern-Volmer plot. The equation of the Stern-Volmer plot is shown in Equation (1).

$$\frac{I_0}{I} = K_{SV}[Q] + 1 \quad (1)$$

where I_0 and I are emission intensities observed in the absence and presence of NO_3^- , respectively, Q is the NO_3^- concentration, and K_{SV} is the Stern-Volmer quenching constant. In the fluorescence sensor, the relative emission intensity (I_0/I) can be defined as the response of the sensor.

Figure 5 shows the Stern-Volmer plots derived from the response of the CN to the concentrations of the NO_3^- added onto CN monitored at three excitation wavelengths, which were 278, 310 and 369 nm. Each Stern-Volmer plot was found to be linear with the concentration of NO_3^- in the range of 300–1800 μM . The linear plots indicated that the emission intensities of the CN at each excitation wavelengths were linear functions to the NO_3^- concentrations,

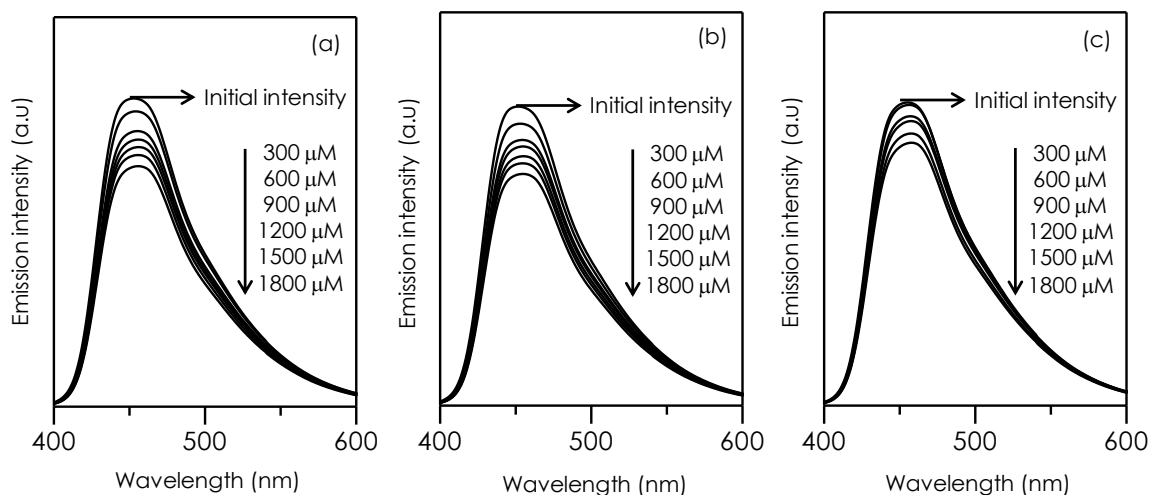


Figure 4 Effect of NO_3^- concentrations on the emission spectra of the CN monitored at excitation wavelengths of (a) 278, (b) 310, and (c) 369 nm

suggesting that the CN can be used as a potential fluorescent sensor for the determination of the NO_3^- . The sensing capability of the CN was revealed from the slopes of the linear plots, which were corresponding to the quenching rate constants (K_{SV}). The K_{SV} value shows the sensitivity of the sensor. The greater the K_{SV} value, the better the sensitivity of the sensor.

It was noted that the K_{SV} value for the emission sites monitored at excitation wavelengths of 278 and 310 nm were similar to each other, which was $2 \times 10^{-4} \mu\text{M}^{-1}$. On the other hand, the emission site monitored at excitation wavelength of 369 nm gave the K_{SV} values of $1 \times 10^{-4} \mu\text{M}^{-1}$. Since the K_{SV} values for the emission sites excited at 278 and 310 nm corresponding to the N=C and the C=O groups, respectively, were two times higher than that of the emission sites excited at 369 nm for the terminal N-C groups, it can be suggested that the N=C and the C=O groups would have more sensitive and stronger interactions with the NO_3^- molecules than the terminal N-C groups. The higher K_{SV} values on the N=C and the C=O groups would be due to the more electropositive N=C and C=O sites than the terminal N-C site, thus, more facilitated the electrostatic interactions between the NO_3^- and the CN sensor. Therefore, it can be proposed that the N=C and the C=O would be the favorable sites to interact with the NO_3^- molecules.

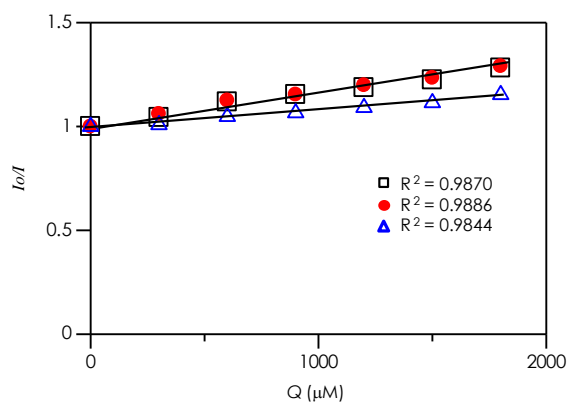


Figure 5 Stern-Volmer plots between the relative emission intensity (response) versus concentrations of NO_3^- molecules added on the CN. The emission spectra were monitored at excitation wavelengths of 278 (□), 310 (●), and 369 (▲) nm

In order to investigate the LOD of the CN, the reproducibility of the CN at the linear range was investigated at four repetitions. Figure 6 shows the reproducibility of the CN sensor at each excitation wavelength and each concentration of the NO_3^- . The error bars showed the SD values. The CN showed good reproducibility with the RSD values in the range of 1.5-5.9, 2.4-7.2 and 2.5-5.9% at each excitation wavelength of 278, 310 and 369 nm, respectively.

The LOD of the CN for the NO_3^- was then determined from the standard deviation of the response at the intercept of the regression line and

the value of the slope, as shown in Equation (2). It was obtained that the LOD value for the CN was 261 μM at excitation wavelength of 278 nm.

$$\text{LOD} = \frac{3 \times \text{SD}}{K_{SV}} \quad (2)$$

The stability of the CN sensor was evaluated by reusing the CN to detect the NO_3^- . After washing and drying processes, the CN was reused and its K_{SV} values were compared to the K_{SV} values of the fresh CN. It was confirmed that the reused CN showed very similar K_{SV} values to those of the fresh CN at all excitation wavelengths. Therefore, it can be concluded that the CN is a stable fluorescence sensor for the detection of the NO_3^- .

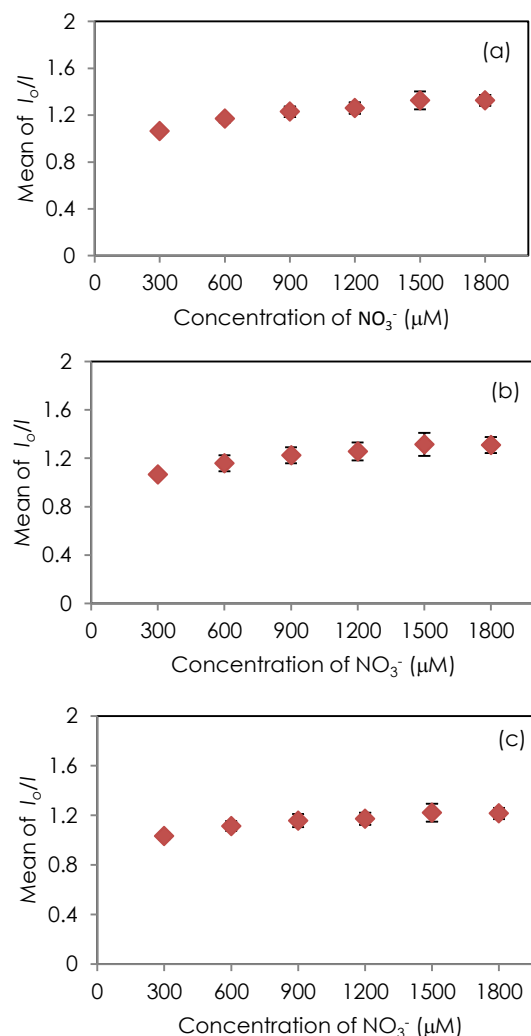


Figure 6 The reproducibility of the CN responses at each NO_3^- concentration excited at (a) 278, (b) 310, and (c) 369 nm. The SD values are shown as error bars

4.0 CONCLUSION

A metal-free CN as a fluorescence sensor has been successfully synthesized, characterized, and applied to detect the NO_3^- . The CN showed a linear detection range at 300–1800 μM . The N=C and the C=O sensing sites would be the favorable sites to interact with the NO_3^- molecules via electrostatic interactions. The CN showed good reproducibility and reusability with limit of detection as low as 261 μM at the N=C sites. All the obtained results indicated that the CN is a potential fluorescence sensor for detection of NO_3^- . Future works should be directed to the fabricated sensor applications for the real samples.

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