



# Article Point-of-Care Testing Kit for the Detection of Hexavalent Chromium by Carbohydrazide-Derived Graphitic Carbon Nitride

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**Abstract:** Hexavalent chromium (Cr(VI)) ions are among the most common hazardous metals that pose a serious risk to human health, causing human carcinogenesis and chronic kidney damage. In this study, a point-of-care testing (POCT) kit is proposed for Cr(VI) ions detection at room temperature. The kit contains a hydrophobic parafilm, a nylon membrane to resist outflow, and a hydrophilic Whatman filter paper suitable for coating the fluorescent graphitic carbon nitride sheet (g-C<sub>3</sub>N<sub>4</sub>). Crystalline, nano-porous, blue-emitting g-C<sub>3</sub>N<sub>4</sub> was produced by pyrolysis utilizing carbohydrazide. The electrostatic interactions between the g-C<sub>3</sub>N<sub>4</sub> and Cr(VI) ions inhibit the fluorescence behavior. The POCT kit can be used for on-site Cr(VI) ion detection dependent upon the blue emission value. The detection limit was attained at 4.64 nM of Cr(VI) ions. This analytical methodology was utilized on real samples from tap, pond, river, and industrial wastewater. This POCT kit can be a useful alternative for on-site detection of Cr(VI) ions.

**Keywords:** point-of-care testing (POCT); carbohydrazide; graphitic carbon nitride sheet (g-C<sub>3</sub>N<sub>4</sub>); fluorescence; hexavalent chromium ions (Cr(VI) ions)



Citation: Maruthupandi, M.; Lee, N.Y. Point-of-Care Testing Kit for the Detection of Hexavalent Chromium by Carbohydrazide-Derived Graphitic Carbon Nitride. *Chemosensors* 2024, 12, 180. https://doi.org/10.3390/ chemosensors12090180

Received: 15 July 2024 Revised: 2 September 2024 Accepted: 3 September 2024 Published: 5 September 2024



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# 1. Introduction

The rapid pace of industrialization has resulted in major environmental issues because of the illegal and irresponsible waste disposal in aqueous systems. Heavy metal contamination of marine and ground aqueous environments poses a major risk to human health [1]. The most common heavy metals that cause human poisoning are arsenic, cadmium, chromium, lead, and mercury. Annually, 2.4 million tons of toxic metal waste, such as chromium, cadmium, zinc, vanadium, arsenic, nickel, mercury, and copper, are produced by the fuel and power industries [2,3].

Chromium is used in various forms, such as dichromate, ferrochrome, and sodium chromate. Hexavalent chromium (Cr(VI)) ions are an important water pollutant, and largely used in boilers as a coating for anticorrosive agents. Chromium waste is produced from industrial processes such as printing, leather tanning, chromium plating, stainless soldering, wood preservation, and pigment production. Chromic acid and Cr(VI) ions are regarded as exceedingly dangerous because even in small amounts, they can cause organ damage, stomach pain, diarrhea, and chronic kidney damage, and they are human carcinogens [4]. The World Health Organization recommends a maximum allowable concentration of 0.05 mg/L for Cr(VI) ions in drinking water [5]. Accordingly, sensitive, cost-effective, and straightforward on-site monitoring of Cr(VI) ions is crucial, especially for minimizing financial impact in developing countries.

Current standard available methods for Cr(VI) ion detection include graphite furnace atomic spectroscopy [6], atomic fluorescence spectroscopy [7], inductively coupled plasma

mass spectrometry [8], flame atomic absorption spectroscopy [9], high-performance liquid chromatography [10], and atomic absorption spectrophotometry [11]. Among these, the fluorescence-based sensors are particularly notable because of their quick reaction time, high sensitivity, selectivity, and easy modification [12]. Fluorescence-based point-of-care testing (POCT) diagnostic kits have recently gained momentum in the analytical sector because of their low cost and ease of use for on-site monitoring.

Graphitic carbon nitride (g-C<sub>3</sub>N<sub>4</sub>) has emerged as a promising material for fluorescencebased sensors due to its unique optical and chemical properties. The g-C<sub>3</sub>N<sub>4</sub> is a novel class of metal-free semiconductor with a high surface area, nanopores, excellent stability, and fluorescence characteristics [13,14]. The polymeric substance g-C<sub>3</sub>N<sub>4</sub> is composed of an s-triazine-based pattern with a carbon-to-nitrogen ratio of 3/4 and a minor quantity of H [15,16]. Urea and its derivatives, such as thiourea and carbohydrazide, are common and cost-effective precursors for synthesizing g-C<sub>3</sub>N<sub>4</sub>, but this usually produces lower yields, around 30–40%. In contrast, derivatives like melamine, cyanamide, dicyandiamide, and guanidine generally result in higher yields of g-C<sub>3</sub>N<sub>4</sub>, around 50–70%. Urea and its derivatives used in g-C<sub>3</sub>N<sub>4</sub> are low-cost materials that offer excellent catalytic performance and high porosity. During the preparation process, large amounts of gases, such as ammonia (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>), are released. The release of these gases during the polymerization of urea and its derivatives into g-C<sub>3</sub>N<sub>4</sub> creates a more porous structure [13,17]. Understanding these differences helps in choosing the best method for large-scale production of g-C<sub>3</sub>N<sub>4</sub>.

Because of its fascinating properties,  $g-C_3N_4$  is being increasingly used in bioimaging [18], photoelectron catalysis [19], drug delivery [20], and fluorescence sensors [21]. Wen Sun et al. developed a tannic acid (TA) and polyethyleneimine (PEI) capped- $g-C_3N_4$ composite for efficient Cr(VI) ions removal from water, demonstrating high adsorption capacity and effective reduction of Cr(VI) ions to Cr(III) [21]. Therefore, we fabricated a fluorescence-based point-of-care testing kit using carbohydrazide-derived  $g-C_3N_4$ . The smartphone-assisted POCT kit was used as a probe for on-site monitoring of Cr(VI) ions.

# 2. Materials and Methods

## 2.1. Reagents and Chemicals

Carbohydrazide (CO(NHNH<sub>2</sub>)<sub>2</sub>,  $\geq$ 98%), potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>,  $\geq$ 99%), Congo red (CR), and chromium(III) oxide (CrO<sub>3</sub>,  $\geq$ 98.5%) were obtained from Sigma-Aldrich, St. Louis, MO, USA. Iron(III) chloride (FeCl<sub>3</sub>), sodium chloride (NaCl), mercury chloride (HgCl<sub>2</sub>), iron(II) sulfate (FeSO<sub>4</sub>), potassium chloride (KCl), ascorbic acid (AA), nickel nitrate (Ni(NO<sub>3</sub>)<sub>2</sub>), zinc acetate (Zn(CH<sub>3</sub>CO<sub>3</sub>)<sub>2</sub>), copper sulfate (CuSO<sub>4</sub>), sodium hydroxide (NaOH,  $\geq$ 99%), and acetic acid (CH<sub>3</sub>COOH,  $\geq$ 99%) were obtained from Fisher Scientific, Waltham, MA, USA. Disodium phosphate (Na<sub>2</sub>HPO<sub>4</sub>,  $\geq$ 95%) and monosodium phosphate (NaH<sub>2</sub>PO<sub>4</sub>,  $\geq$ 99%) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Whatman filter paper (460 mm × 570 mm), nylon membrane filters (pore size: 0.22 µm), and parafilm were purchased from Sigma-Aldrich, St. Louis, MO, USA. A phosphate buffer (0.2 M) with pH ranging from 4.0 to 11.0 was prepared by mixing NaH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub>, and NaOH and CH<sub>3</sub>COOH was used to adjust the pH.

## 2.2. Synthesis of g- $C_3N_4$

Scheme 1 shows a simplified representation of the preparation of  $g-C_3N_4$  from carbohydrazide via pyrolysis. First, 1.5 g of carbohydrazide powder was placed in a silica crucible. Subsequently, the silica crucible was kept in muffle furnace for pyrolysis for 3 h at 550 °C (temperature rate: 4 °C/min) in an air atmosphere. Then, the silica crucible was allowed to reach room temperature (RT) to produce a yellow crystalline powder ( $g-C_3N_4$  yield: 24.52%). Subsequently, the  $g-C_3N_4$  powder was ground well using a granite mortar and pestle. Then, a  $g-C_3N_4$  suspension solution was prepared by dissolving 0.1 g of  $g-C_3N_4$  in 10 mL of (15%) ethanol in double distilled water (DDW). The suspension solution (pH 6.5) was sonicated at 200 W and 40 ± 3 kHz at 26 °C for 10 min. Finally, the

unreacted substances were removed from the g- $C_3N_4$  suspension solution by centrifugation at 10,000 rpm for 10 min. This process of sonication and centrifugation was repeated three times. The precipitate was removed, and the resulting 0.03 mL of purified g- $C_3N_4$  suspension solution (21.5 mg/mL) mixed with 2.97 mL of phosphate buffer at pH 8 was used for sensor applications.



**Scheme 1.** Schematic illustration of the synthesis of carbohydrazide derived g-C<sub>3</sub>N<sub>4</sub> by pyrolysis method.

#### 2.3. Characterizations

Absorption spectral studies were performed in an ultraviolet-visible (UV-vis) spectrophotometer lambda-25 (Perkin Elmer, Waltham, MA, USA). Emission spectral studies were performed by spectrofluorometry FP-8200 (JASCO, Hachioji, Tokyo, Japan). Powder X-ray diffraction (XRD) study was performed using XRD-Rigaku unit with ( $\lambda = 1.5406$ )  $CuK\alpha$ , Ni-filtered radiation (Neu-Isenburg, German). Transmission electron microscopy (TEM) images were captured using a JEOL-2100 (Peabody, MA, USA) microscope at 200 kV. Fourier-transform infrared spectrophotometer (JASCO 6300, Hachioji, Tokyo, Japan) was used to record the Fourier-transform infrared spectroscopy (FT-IR) spectrum of  $g-C_3N_4$ . A confocal Raman spectroscope (Alpha300R, WiTech GmbH, Remscheid, Germany) was used to perform the Raman spectrum studies. The X-ray photoelectron spectra were recorded using an X-ray photoelectron spectroscopy (XPS) unit (Shimadzu Axis 1165, Nakagyo-ku, Kyoto, Japan) with Al K radiation energy at 80 eV. Nitrogen (N<sub>2</sub>) adsorption/desorption isotherms and pore size distribution were measured using the Brunauer-Emmett-Teller (BET) method with a Quantachrome-ASiQwin, Boynton Beach, Florida system (version 3.0) at 77 K. Simultaneous thermal analyzer (NETZSCH-STA 449 F5, Selb, Germany) was used to analyze weight percent composition of the g-C<sub>3</sub>N<sub>4</sub>. The analysis was carried out in a nitrogen (N<sub>2</sub>) atmosphere, covering a temperature range from 30  $^\circ$ C to 1300  $^\circ$ C, with a temperature rate of 10 °C/min. The POCT kit was prepared using a hydraulic hot press machine from PCI Analytics Pvt. Ltd., Thane, India.

## 2.4. Detection of Cr(VI) Ions Using the Spectrofluorometric Method

The spectrofluorometric method used for detection of Cr(VI) ions was performed according to the following procedure. In a 3.5 mL quartz cuvette, 0.03 mL of a purified g-C<sub>3</sub>N<sub>4</sub> suspension solution (21.5 mg/mL) and 2.97 mL of phosphate buffer (pH 8) were added simultaneously. The final concentration of g-C<sub>3</sub>N<sub>4</sub> in the cuvette was calculated to be 0.217 mg/mL. This concentration of g-C<sub>3</sub>N<sub>4</sub> suspension solution, as mentioned above, was used for all absorbance and fluorescence spectral studies. Different known Cr(VI) ions concentrations were added to the cuvette mixture, and their emission variations were monitored by fluorescence spectrometry at 2 min intervals. The LOD of Cr(VI) ions was calculated using Equation (1):

$$LOD = 3S/m \tag{1}$$

where "LOD" is the detection limit of Cr(VI) ions; "S" represents the standard deviation of the emission value of g-C<sub>3</sub>N<sub>4</sub>; "m" is the slope of the calibration curve.

# 2.5. Real Sample Analysis

Pond and river samples were collected from the Urapakkam Pond and the Palar River in Tamil Nadu, India. Tap water samples were collected from the university campus, and industrial wastewater samples were collected from the leather industry in Chromepet, Chennai, India. Whatman filter paper was utilized for collecting the dust particles from all of the real samples. In addition, 1.2 and 1.6  $\mu$ M of Cr(VI) ions were spiked into the above-mentioned water samples to detect Cr(VI) ions.

## 2.6. Fabrication of the POCT Kit

Figure S1 displays the essential materials used to prepare the POCT kit. As starting materials, a nylon membrane, parafilm, cross-sectional structures with cut nylon membrane and parafilm, cross-sectional structures printed on Whatman filter paper, and hydraulic pellet pressure were used for the fabrication of a double-sided hydrophobic POCT kit. We printed a cross-sectional structure (sampling circle = 0.5 cm, line length = 1 cm, line width = 0.1 cm) on the Whatman filter paper (460 mm × 570 mm) using an HP Laser Jet Pro M126 printer, Palo Alto, CA, USA.

Figure S2 illustrates the six-step fabrication process for the double-sided hydrophobic paper-based POCT kit. (1) Preparation of bottom hydrophobic sheet: we prepared the bottom hydrophobic layer using nylon and parafilm by thermally bonding at 45 °C/10 s using a hydraulic hot press machine. (2) Preparation of hydrophilic paper: cross-sectional structures were printed on Whatman filter paper for use at sampling sites. (3) Preparation of upper hydrophobic sheet: the upper hydrophobic layer was developed utilizing cross-sectional structures, cut parafilm, and nylon by thermally bonding at 45 °C/10 s. (4) Arrangement of POCT kit: the bottom hydrophobic sheet, hydrophilic filter paper, and upper hydrophobic sheet were sandwiched by thermally bonding at 45 °C/10 s. (5) Reduce thicknesses of POCT kit: the POCT kit thicknesses was reduced using a hydraulic hot-press machine (50 kg/cm<sup>3</sup>). (6) Preparation of g-C<sub>3</sub>N<sub>4</sub>-coated paper-based POCT kit: 100  $\mu$ L of g-C<sub>3</sub>N<sub>4</sub> suspension solution was added to the center well.

#### 2.7. Detection of Cr(VI) Ions Using Smartphone-Assisted POCT Kit

A POCT kit was used to detect Cr(VI) ions. Various concentrations of Cr(VI) ions were injected into different wells of the POCT kit, subsequently capturing photographs under UV light using an OPPO F15 smartphone camera (48 megapixels). The captured photographs were uploaded to a Color Picker app (version 7.2.4, Mikhail Gribanor, Kryvyi Rih, Ukraine) to compute the luminance value (%). The concentration of Cr(VI) ions was determined based on the luminance values (%).

#### 3. Results and Discussion

## 3.1. Absorption and Emission Behaviors of $g-C_3N_4$

Figure 1a shows the absorption and emission spectra of the  $g-C_3N_4$  suspension solution. The absorption hump was observed at 400 nm, and the maximum fluorescence intensity was observed at 418 nm at an excitation wavelength of 310 nm. The absorption peak results were from the excitation of electrons in the valence to conduction bands of  $g-C_3N_4$  [22]. The  $g-C_3N_4$  suspension solution was observed to be blue under UV light irradiation and pale yellow under daylight (Figure 1a, inset). Figure S3 shows the fluorescence spectrum of the starting material (carbohydrazide), which does not show any emission peaks.

Carbohydrazide emission was not observed under UV light (Figure S3; inset). The CIE chromaticity diagram of  $g-C_3N_4$  is displayed in Figure S4. The recorded coordinates of 0.156, 0.159 confirm the blue-emitting nature of  $g-C_3N_4$ . Figure 1b shows the fluorescence spectra of the  $g-C_3N_4$  suspension solution at excitation wavelengths in the range of 280–370 nm. The emission intensity redshifts are enhanced in the excitation wavelength range of 280–310 nm. The emission intensity decreased dramatically with the red shift in the excitation wavelength range of 310–370 nm. Maximum fluorescence emission was observed at 418 nm at an excitation wavelength of 310 nm. Figure S5 shows the normalized fluorescence spectra of the  $g-C_3N_4$  suspension solution at excitation wavelengths in the range from 280–370 nm; the normalized spectra were also red-shifted. The  $g-C_3N_4$  fluo-

rescence properties originate from the electronic band structure, surface defects, porous nature, and electron relocalization [13].



**Figure 1.** (a) Absorption and emission spectra of g- $C_3N_4$  suspension solution (inset: photographs of g- $C_3N_4$  suspension solution under daylight and UV light); (b) 3D plot of the fluorescence spectra of g- $C_3N_4$  obtained with different excitation wavelengths.

# 3.2. FT-IR Vibrational Spectrum and TGA Analysis of g-C<sub>3</sub>N<sub>4</sub>

Figure 2a depicts the FT-IR spectrum of g-C<sub>3</sub>N<sub>4</sub>. The sharp peak at 810 cm<sup>-1</sup> represents the unique vibration of the triazine ring. A vibrational mode of cross-linked heptazine deformation is observed at 866 cm<sup>-1</sup>. The –C=N stretching vibration mode is responsible for the peaks at 1690 and 1637 cm<sup>-1</sup>; the aromatic, –C–N stretching, and vibration modes have peaks at 1459, 1412, and 1387 cm<sup>-1</sup>, respectively. The stretching mode of the –N–H bond was attributed to the broad peaks at 3176 and 3060 cm<sup>-1</sup> [23]. The structure of g-C<sub>3</sub>N<sub>4</sub> was confirmed based on the functional groups, such as –N–H, –C–N, and –C=N, and the triazine ring. Figure S6 shows the TGA curve of g-C<sub>3</sub>N<sub>4</sub>, which is used to confirm the content and purity of the g-C<sub>3</sub>N<sub>4</sub> material. The TGA spectrum of g-C<sub>3</sub>N<sub>4</sub> shows three distinct weight decompositions. The first decomposition occurs from 30 °C to 140 °C, resulting in a 5% weight loss due to the evaporation of H<sub>2</sub>O. The second stage, between 144 °C and 590 °C, leads to another 5% weight loss, likely corresponding to the decomposition of melem (intermediate). Finally, from 595 °C to 740 °C, there is a 90% weight loss, which is attributed to the decomposition of g-C<sub>3</sub>N<sub>4</sub> into CO<sub>2</sub> and graphite [24]. These results indicate a high purity level of g-C<sub>3</sub>N<sub>4</sub>, with 90% of the material being g-C<sub>3</sub>N<sub>4</sub>.



**Figure 2.** (a) FT-IR spectrum of g-C<sub>3</sub>N<sub>4</sub> and (b) XRD pattern of g-C<sub>3</sub>N<sub>4</sub>.

3.3. Morphology, Shape, and Size Analysis of g-C<sub>3</sub>N<sub>4</sub>

The XRD pattern of  $g-C_3N_4$  is displayed in Figure 2b. Figure 2b shows two diffraction peaks at 27.4° (002) and 13.3° (100). The "d" space value due to the stacking of the conjugated aromatic graphite system was calculated as 0.33 nm (002) and the distance

of the nitride pores as 0.67 nm (100) [25]. The XRD results confirmed that  $g-C_3N_4$  has a crystalline nature and interlayer-stacked networks [26]. The "d" space value was calculated using Bragg's law based on X-ray diffraction (XRD) data. The interplanar spacing "d" was determined by Equation (2):

$$d = n \times \lambda/2 \times \sin \theta \tag{2}$$

where "d" is the interplanar spacing; "n" is the order of reflection (1); " $\lambda$ " is the wavelength of the incident X-rays (0.15418); " $\theta$ " is the Bragg angle, 27.4° (002) and 13.3° (100). The BET gas sorptometry measurements results are shown in Figures S7 and S8. Figure S7 illustrates the nitrogen adsorption–desorption isotherms of g-C<sub>3</sub>N<sub>4</sub>, indicating that the prepared g-C<sub>3</sub>N<sub>4</sub> has a large surface area of 98.05 m<sup>2</sup>/g. As depicted in Figure S8, g-C<sub>3</sub>N<sub>4</sub> exhibits a range of pore sizes, with a notable pore size of 25.856 nm and a pore volume of 0.124 m<sup>3</sup>/g. These results confirm the presence of surface defects and holes in the g-C<sub>3</sub>N<sub>4</sub> structure.

Figure 3 shows TEM images of g- $C_3N_4$ . The TEM images show thin, wrinkled, and nano-porous structures, which can result in higher specific surface coverage and more reactive sites. The average pore size was calculated to be  $21.0 \pm 0.7$  nm using Image J-1.54a. The TEM image confirms the wrinkled and nano-porous structure of g- $C_3N_4$  [26].



Figure 3. HR-TEM images of g-C<sub>3</sub>N<sub>4</sub> at (a) 100 nm and (b) 200 nm magnification.

3.4. Raman Vibrational and XPS Spectral Analysis of g-C<sub>3</sub>N<sub>4</sub>

Figure 4a shows the Raman spectrum of  $g-C_3N_4$ . The Raman spectrum exhibits three vibrational peaks at 1541, 1360, and 702 cm<sup>-1</sup>. The "G" band appears at 1541 cm<sup>-1</sup>, and the "D" band appears at 1360 cm<sup>-1</sup>, corresponding to  $sp^2$  carbon and  $sp^3$  carbon, respectively. The  $sp^3$  carbon represents more surface defects and a porous structure of  $g-C_3N_4$ . The significant peak at 702 cm<sup>-1</sup> indicates the vibrational mode of the C-N heterocycles. The Raman results confirm that  $g-C_3N_4$  has a porous structure [27].

Figure 4b shows the XPS survey spectrum of  $g-C_3N_4$ . The XPS results were used to determine the element composition and structure of  $g-C_3N_4$ . The survey spectra revealed three peaks at 534, 402, and 291 eV, which correspond to the O, N, and C chemical species, respectively [28]. In Figure 4c, the high-resolution C1s spectra of the three carbon species are observed at 284.7, 288.8, and 290 eV. The lower binding energy peak (284.7 eV) can be attributed to the carbon double bond (C=C) in graphite [29]. The s-triazine and pyridine-like aromatic (N–C=N) ring structure is responsible for the binding energy at 288.8 eV [30]. The graphitic-like nitrogen structure (N trigonally linked with three sp<sup>2</sup> C atoms) has a higher binding energy of 290 eV [28]. Figure 4d shows the high-resolution spectra of N1s and the three nitrogen species at 398.3, 399.7, and 401.2 eV. Pyrrolic sp<sup>2</sup> nitrogen (C=NH) has a lower binding energy, observed at 398.3 eV [30]. The peak observed at 401.2 eV



was identified as tertiary nitrogen (N–(C)<sub>3</sub>) groups [29]. A very small number of oxygen groups, such as hydroxyl and carboxyl groups, were observed at 534 eV. The XPS results confirmed the  $g-C_3N_4$  structure.

**Figure 4.** (a) Raman spectrum of g- $C_3N_4$ ; (b) X-ray photoelectron spectroscopy survey spectrum of g- $C_3N_4$ ; (c) high-resolution spectrum of C1s; (d) high-resolution spectrum of N1s species.

## 3.5. Effect of pH and Time

Figure S9a shows the fluorescence spectra of g- $C_3N_4$  in the pH range of 4–11. The emission intensity increases with pH from 3 to 8, reaching a peak at pH 8. At acidic pH, the lower emission intensity is attributed to protonation at nitrogen sites in the heptazine rings, which can cause partial decomposition or dissolution. Beyond pH 8, the emission intensity significantly decreases due to the reaction of excess –OH ions with carbon-nitrogen bonds, leading to material degradation. The high emission intensity observed at pH 8 is due to an enhanced negative surface charge, which improves cation (Cr(VI) ions) adsorption [32]. Therefore, we set the pH to 8 for subsequent experiments (Figure S9b). Figure S10 shows the incubation time for the interaction of g- $C_3N_4$  with Cr(VI) ions. The fluorescence intensity of the g- $C_3N_4$  and Cr(VI) ions mixture was observed over time intervals from 1 to 10 min. The relative fluorescence intensity increased from 1 to 2 min and remained constant after 2 min. Therefore, an optimal incubation time of 2 min was established for the determination of Cr(VI) ions.

## 3.6. Quantum Yield of g-C<sub>3</sub>N<sub>4</sub>

A relative comparison methodology was utilized for calculating the quantum yield of  $g-C_3N_4$ . Quinine sulfate was used as a reference for the quantum yield measurements in this study. Seven different concentrations of quinine sulfate and  $g-C_3N_4$  suspension solution were prepared. The absorption and fluorescence spectra were recorded in the quinine sulfate and  $g-C_3N_4$  samples. Figures S11 and S12 show the relative absorbance as a function

of the integrated fluorescence intensity of quinine sulfate and  $g-C_3N_4$ , respectively. The fluorescence quantum yield of  $g-C_3N_4$  was calculated using the following Equation (3) [33].

$$QY_{gCN} = F_{gCN} \times A_s \times QY_s / F_s \times A_{gCN}$$
(3)

where " $A_{gCN}$ " is the excitation wavelength of g- $C_3N_4$  (310 nm); " $F_{gCN}$ " is the integrated fluorescence emission area of g- $C_3N_4$  (414,321); "QY<sub>s</sub>" is the quantum yield of the standard quinine sulfate (54%); " $A_s$ " is the excitation wavelength of quinine sulfate (320 nm); " $F_s$ " is the integrated fluorescence area of quinine sulfate (1,266,010); and "QY<sub>gCN</sub>" is the fluorescence quantum yield of g- $C_3N_4$ , calculated as 18.24%.

## 3.7. Absorption Behavior of g-C<sub>3</sub>N<sub>4</sub> and Cr(VI) Ions Mixture

Figure 1a shows the absorption spectra of  $g-C_3N_4$  and an absorption band at 400 nm. The g-C<sub>3</sub>N<sub>4</sub> suspension solution was pale yellow under daylight (Figure 1a, inset). Figure S13 presents the absorption spectrum of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (Cr(VI) ions). Absorption peaks are observed at 257 and 352 nm, and the color of the suspension solution is dark yellow (Figure S13, inset) [34]. Figure S14 displays the absorption spectrum of  $g-C_3N_4$  and a 5.2  $\mu$ M mixture of Cr(VI) ions with absorption peaks at 247, 302, 435, and 585 nm. A light green color is observed in the g-C<sub>3</sub>N<sub>4</sub> and Cr(VI) ions mixture (Figure S14, inset). These g-C<sub>3</sub>N<sub>4</sub> and 5.2  $\mu$ M mixture absorbance spectra and color results clearly matched with chromium (III) oxide nanoparticles (Cr<sub>2</sub>O<sub>3</sub> NPs) [35,36]. The Cr(VI) ions are to reduce trivalent chromium (Cr(III)) ions because of catalytic, nano-porous, and electron-rich g-C<sub>3</sub>N<sub>4</sub>.

## 3.8. Sensing of Cr(VI) Ions by Spectrofluorometry

Figure 5a shows the emission spectra of the g-C<sub>3</sub>N<sub>4</sub> suspension solution with various Cr(VI) ions concentrations. The fluorescence intensity maximum of the g-C<sub>3</sub>N<sub>4</sub> suspension solution is observed at 418 nm. The emission intensity was quenched with the injection of 0.4  $\mu$ M Cr(VI) ions. Increasing the amount of Cr(VI) ions to 5.2 from 0.8  $\mu$ M led to a decrease in the emission intensity.



**Figure 5.** (a) Emission spectra of g- $C_3N_4$  in the presence of various concentrations of Cr(VI) ions (0.4 to 5.2  $\mu$ M); inset: photograph of g- $C_3N_4$  (negative) and g- $C_3N_4$  in the presence of 5.2  $\mu$ M of Cr(VI) ions (positive) under UV light. (b) Linear plot: fluorescence intensity vs. concentration of Cr(VI) ions (M).

The Figure 5a inset shows photographs of the g- $C_3N_4$  suspension solution (negative) and a mixture of Cr(VI) ions and g- $C_3N_4$  (positive) under UV light. Figure 5b shows a linear plot of fluorescence intensity as a function of Cr(VI) ions concentration. The detection limit was calculated to be 4.64 nM, attaining good linearity, ranging from 400 nM to 5.2  $\mu$ M of Cr(VI) ions.

#### 3.9. Morphology, Shape, and Size Analysis of the g-C<sub>3</sub>N<sub>4</sub> and Cr(VI) Ions Mixture

Figure 6a,b show TEM images of g- $C_3N_4$  in the presence 1.2  $\mu$ M of  $K_2Cr_2O_7$  (Cr(VI) ions). The TEM images revealed the formation of  $Cr_2O_3$  nanoparticles (Cr(III)) on the surface of the porous g- $C_3N_4$  structure. The average pore size of the aggregated g- $C_3N_4$  was measured to be  $60 \pm 7$  nm, while the  $Cr_2O_3$  nanoparticles had an average size of 23.8  $\pm$  8 nm [37]. Notably, the pore size of the g- $C_3N_4$  in the aggregate was approximately 2.1 times larger than that of the free g- $C_3N_4$ . These results suggest that the  $Cr_2O_3$  nanoparticles are adsorbed onto the g- $C_3N_4$  due to its catalytic and porous properties.



**Figure 6.** TEM images of g-C<sub>3</sub>N<sub>4</sub> in the presence of 1.2  $\mu$ M Cr(VI) ions at (**a**) 100 nm and (**b**) 200 nm magnification.

#### 3.10. Sensing Mechanism

Scheme 2 shows a schematic representation of the possible mechanism of the Cr(VI) ions detection using the g-C<sub>3</sub>N<sub>4</sub> probe. The XPS, FT-IR spectra, XRD patterns, and HR-TEM images clearly demonstrate that the synthesized g-C<sub>3</sub>N<sub>4</sub> has a thin, wrinkled structure and is extremely porous. The highly porous structure, along with the catalytic and electron-rich nitrogen groups of g-C<sub>3</sub>N<sub>4</sub>, facilitates redox reactions with Cr(VI) ions, thereby inhibiting its fluorescence behavior. The absorbance spectra results (Section 3.7) clearly indicate that K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (Cr(VI) ions) are reduced to Cr<sub>2</sub>O<sub>3</sub> nanoparticles (Cr(III) ions) in the presence of a g-C<sub>3</sub>N<sub>4</sub> suspension solution. Additionally, due to the oxidation properties of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, the g-C<sub>3</sub>N<sub>4</sub> undergoes oxidation, leading to the formation of functional groups (e.g., –COOH, –OH, –C=O) at the open ends or defect sites of the g-C<sub>3</sub>N<sub>4</sub> structure. The TEM results (Section 3.9) further confirm the formation of Cr<sub>2</sub>O<sub>3</sub> nanoparticles [38]. Figure S15 shows photographs of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (Cr(VI) ions) and a mixture of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and ascorbic acid (AA). We observed that K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> appears as a yellow color, while the mixture of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and AA appears green. This change in color confirms that ascorbic acid (reducing agent) effectively reduces Cr(VI) ions to Cr(III), likely forming Cr<sub>2</sub>O<sub>3</sub> nanoparticles.

Moreover, we analyzed the spectroscopic mechanism for the sensing of Cr(VI) ions. Figure S16 shows the emission and excitation spectra of  $g-C_3N_4$  at wavelengths in the range of 332–577 nm and 217–470 nm. Figure S16 shows the absorption spectrum of Cr(VI) ions ( $K_2Cr_2O_7$ ) in the range of 311–500 nm. The broad absorption spectrum of the Cr(VI) ions (quencher) significantly overlays with the emission and excitation spectra of  $g-C_3N_4$  (fluorophore). This spectral overlap process inhibits the fluorescence behavior of  $g-C_3N_4$ . This spectral mechanism is known as the inner filter effect (IFE) [39,40].

Figure S17 presents the absorbance spectrum of Congo red (CR) alongside the excitation and emission spectra of g- $C_3N_4$ . The absorbance spectrum of Congo red extends from 280 to 598 nm. The broad absorption band of Congo red, which acts as a quencher, significantly overlaps with the excitation and emission spectra of g- $C_3N_4$ , a fluorophore. This extensive spectral overlap leads to the suppression of the fluorescence behavior of g- $C_3N_4$ . Figure S18 shows the emission spectra of g- $C_3N_4$  in the presence of 4  $\mu$ M and 20  $\mu$ M CR. The inset of Figure S18 features a photograph of g-C<sub>3</sub>N<sub>4</sub> and g-C<sub>3</sub>N<sub>4</sub> with 4  $\mu$ M and 20  $\mu$ M CR under UV light. Upon adding CR to the g-C<sub>3</sub>N<sub>4</sub> solution, the fluorescence is slightly quenched. While the absorbance spectra of Congo red and Cr(VI) ions occur at similar wavelengths, Cr(VI) ions are more effective at inducing an inner filter effect than Congo red.



**Scheme 2.** Schematic representation of the possible mechanism of detection of Cr(VI) ions using the g-C<sub>3</sub>N<sub>4</sub> probe.

## 3.11. Selectivity Study

Figure S19 shows photographs of 400  $\mu$ M solutions of NaCl, KCl, FeCl<sub>3</sub>, FeSO<sub>4</sub>, CuSO<sub>4</sub>, HgCl<sub>2</sub>, Zn(CH<sub>3</sub>CO<sub>2</sub>)<sub>2</sub>, Ni(NO<sub>3</sub>)<sub>2</sub>, CrO<sub>3</sub>, and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> dissolved in a phosphate buffer at pH 8. All the metal salts are fully soluble in the phosphate buffer and were used for an interference study. Figure 7a shows the results of the selectivity study using common metal ion interferences, including Na<sup>+</sup>, K<sup>+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Fe<sup>2+</sup>, Hg<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, and Cr<sup>3+</sup> ions. Figure 7b shows a photograph of the selectivity under UV light. The addition of 400  $\mu$ M (333-fold) concentrations of common metal ion interferences did not affect the relative fluorescence intensity and color of g-C<sub>3</sub>N<sub>4</sub>. Even after the addition of a 400  $\mu$ M metal cation mixture to g-C<sub>3</sub>N<sub>4</sub>, the relative fluorescence intensity and color remained unchanged. The injection of 1.2  $\mu$ M Cr(VI) ions into g-C<sub>3</sub>N<sub>4</sub> induced a change in fluorescence intensity and color shift from blue to colorless. These interference results indicate that g-C<sub>3</sub>N<sub>4</sub> acts as a selective detection material for Cr(VI) ions.



**Figure 7.** (a) Bar diagram of relative emission intensity of  $g-C_3N_4$  based on the addition of  $Na^+$ ,  $K^+$ ,  $Fe^{3+}$ ,  $Cu^{2+}$ ,  $Fe^{2+}$ ,  $Hg^{2+}$ ,  $Zn^{2+}$ ,  $Ni^{2+}$ , and  $Cr^{3+}$ , mixed cations, and Cr(VI) ions; (b) corresponding photographs under UV light.

During the redox reaction,  $K_2Cr_2O_7$  (Cr(VI) ions) is reduced to  $Cr_2O_3$  nanoparticles, while g-C<sub>3</sub>N<sub>4</sub> is simultaneously oxidized, forming functional groups within the g-C<sub>3</sub>N<sub>4</sub> structure. The comparatively lower oxidation properties of Cr(III) (such as Cr<sub>2</sub>O<sub>3</sub>) and other interference mean that it does not significantly disrupt the emission properties of g-C<sub>3</sub>N<sub>4</sub>, which explains why Cr(III) and other interference does not interfere with the fluorescence.

## 3.12. Real Samples Analysis

Table 1 displays the results demonstrating the practicability of Cr(VI) ions detection in real samples. Tap, pond, river water, and industrial wastewater samples were used for the real sample analysis, and Cr(VI) ions were not identified. However, we spiked the 1.2 and 1.6  $\mu$ M Cr(VI) ions in the real samples used to identify Cr(VI) ions. Depending upon the emission intensity, the acceptable recoveries were determined to be in the range of 95.6–103.3%. These results indicate that the g-C<sub>3</sub>N<sub>4</sub> suspension solution can sense Cr(VI) ions in tap water, ponds, rivers, and industrial wastewater samples.

Real Sample	Cr(VI) Ions Spiked (µM)	Cr(VI) Ions Found (μM)	Recovery (%)
Tap water	0	0	0
	1.2	$1.19\pm0.03$	$99.2 \pm 0.3$
	1.6	$1.55\pm0.02$	$96.87\pm0.12$
Pond water	0	0	0
	1.2	$1.24\pm0.03$	$103.3\pm0.2$
	1.6	$1.58\pm0.01$	$98.7\pm0.3$
River water	0	0	0
	1.2	$1.18\pm0.03$	$98.33 \pm 0.14$
	1.6	$1.54\pm0.02$	$96.25\pm0.13$
Industry wastewater	0	0	0
	1.2	$1.22\pm0.04$	$101.6\pm0.3$
	1.6	$1.53\pm0.05$	$95.6\pm0.4$

Table 1. Real sample analysis.

## 3.13. Smartphone-Assisted POCT Kit for On-Site Detection of Cr(VI) Ions

Materials and Methods Section 2.6 provides details regarding the fabrication of the g-C<sub>3</sub>N<sub>4</sub>-coated POCT kit. Figure 8a shows a photograph of the g-C<sub>3</sub>N<sub>4</sub>-coated POCT kit used to detect Cr(VI) ions. The g-C<sub>3</sub>N<sub>4</sub>-coated POCT kit contained five wells emitting a blue color under UV light. Subsequently, we added a range of 0.4 to 5.2  $\mu$ M concentrations of Cr(VI) ions to the POCT kit. The blue fluorescence intensity decreased as the concentration of Cr(VI) ions increased. Figure 8b shows a screenshot of the luminance measurement using a smartphone Color Picker app. Figure 8c shows a linear plot of the luminance value (%) as a function of cncentration Cr(VI) ions. Based on the linearity plot, the concentration of Cr(VI) ions in spiked tap water was determined (Figure 8a,c). The smartphone-assisted POCT kit could be an alternate to expensive methods for the on-site detection of Cr(VI) ions.

Figure S20 shows the POCT kit used to investigate the effects of starting materials on the kit. Starting components such as paper, water, carbohydrazide (CHD), and g-C<sub>3</sub>N<sub>4</sub> were added to the relevant POCT kit under UV light. We noticed that only the g-C<sub>3</sub>N<sub>4</sub>-filled well emitted a blue color. Then, 100  $\mu$ L of 5.2  $\mu$ M Cr(VI) ions were added to the middle well, and the well was automatically moved to the other four wells. The Cr(VI) ions reacted with g-C<sub>3</sub>N<sub>4</sub> only; they did not react with paper, water, or CHD. Figure S21 shows the effect of interference using a g-C<sub>3</sub>N<sub>4</sub>-coated POCT kit. The five-well POCT kit preparation procedure was used to develop a twelve-well POCT kit. We added 15  $\mu$ L of g-C<sub>3</sub>N<sub>4</sub> to each of the wells in the twelve-well POCT kit, and blue color emission was observed under UV light. We added 15  $\mu$ L of common metal ions to each well, including Na<sup>+</sup>, K<sup>+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Fe<sup>2+</sup>, Cr(IV) ions, Hg<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Cr<sup>3+</sup>, and mixed cations (mcs). Interestingly, only the color in the Cr(VI) ions well disappeared, whereas the other metal ions did not affect the



color. Overall, the results show that the smartphone-assisted  $g-C_3N_4$ -coated POCT kit acts as a good probe for the on-site and selective detection of Cr(VI) ions.

**Figure 8.** (a) Photograph of g-C<sub>3</sub>N<sub>4</sub>-coated POCT kit used for detection of Cr(VI) ions (0.4–5.2  $\mu$ M); (b) screenshot of the Color Picker app used to measure luminance; (c) linearity plot: luminance (%) vs. concentration of Cr(VI) ions.

## 4. Conclusions

In this study, we developed a POCT kit for the on-site detection of Cr(VI) ions at RT. The POCT kit was fabricated using hydrophobic parafilm, a nylon membrane to resist outflow, and hydrophilic Whatman filter paper suitable for the coating of fluorescent g- $C_3N_4$ . The g- $C_3N_4$  suspension solution was synthesized from carbohydrazide by pyrolysis and characterized using several techniques, such as fluorescence, UV-Vis, FT-IR, XPS, XRD, Raman spectroscopy, and HR-TEM. The synthesized g- $C_3N_4$  suspension solution exhibited a nano-porous crystalline structure and blue emission, with the emission intensity detected at 418 nm. When Cr(VI) ions were added to the g- $C_3N_4$  suspension solution, a redox reaction occurred between the g- $C_3N_4$  and Cr(VI) ions, leading to the inhibition of the fluorescence behavior of g- $C_3N_4$ . The POCT kit can be used for the on-site detection of Cr(VI) ions based on changes in blue emission. The detection limit was attained at 4.64 nM for the linear range of 0.4–5.2  $\mu$ M of Cr(VI) ions. We applied this methodology to real samples from tap water, ponds, rivers, and industrial wastewaters. The novel POCT kit is expected to provide an alternate to other expensive analytical systems for detecting Cr(VI) ions on-site.

**Supplementary Materials:** The following supporting information can be downloaded at: https:// www.mdpi.com/article/10.3390/chemosensors12090180/s1, Figure S1: Starting materials for the preparation of the POCT kit; Figure S2: Schematic representation of the preparation of the POCT kit; Figure S3: Fluorescence spectrum of carbohydrazide (inset: photograph of carbohydrazide under UV light); Figure S4: Chromaticity diagram of g-C<sub>3</sub>N<sub>4</sub>; Figure S5: Normalized fluorescence spectra of g-C<sub>3</sub>N<sub>4</sub> obtained at different excitation wavelengths; Figure S6: Thermogravimetric (TGA) curve of g-C<sub>3</sub>N<sub>4</sub>; Figure S7: Nitrogen adsorption–desorption isotherms of g-C<sub>3</sub>N<sub>4</sub>; Figure S8: Pore size distribution of g-C<sub>3</sub>N<sub>4</sub>; Figure S9: (a) Fluorescence spectra of g-C<sub>3</sub>N<sub>4</sub> across a pH range from 4 to 11, (b) Bar diagram depicting the relationship between pH and fluorescence intensity of g-C<sub>3</sub>N<sub>4</sub>; Figure S10: Effect of time: relative fluorescence intensity of g-C<sub>3</sub>N<sub>4</sub> and 1.2  $\mu$ M Cr(VI) ions mixture vs time; Figure S11: Integrated fluorescence intensity of quinine sulfate vs relative absorbance; Figure S12: Integrated fluorescence intensity of g-C<sub>3</sub>N<sub>4</sub> vs relative absorbance; Figure S13: Absorption spectrum of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (Cr(VI) ions) (inset: photograph); Figure S14: Absorption spectrum of g-C<sub>3</sub>N<sub>4</sub> in the presence 5.2  $\mu$ M of Cr(VI) ions mixture (inset: photograph); Figure S15: Photographs of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and mixture of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and ascorbic acid (AA); Figure S16: Absorbance spectrum of Cr(VI) ions and excitation and emission spectra of g-C<sub>3</sub>N<sub>4</sub>; Figure S17: Absorbance spectrum of Congo red (CR) and excitation and emission spectra of g-C<sub>3</sub>N<sub>4</sub>; Figure S18: Emission spectra of g-C<sub>3</sub>N<sub>4</sub> in the presence of 4 and 20  $\mu$ M CR (inset: photograph of g-C<sub>3</sub>N<sub>4</sub> and g-C<sub>3</sub>N<sub>4</sub> in the presence of 4 and 20  $\mu$ M CR (inset: photographs of 400  $\mu$ M solutions of NaCl, KCl, FeCl<sub>3</sub>, FeSO<sub>4</sub>, CuSO<sub>4</sub>, HgCl<sub>2</sub>, Zn(CH<sub>3</sub>CO<sub>3</sub>)<sub>2</sub>, Ni(NO<sub>3</sub>)<sub>2</sub>, CrO<sub>3</sub>, and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> dissolved in a phosphate buffer with a pH of 8; Figure S20: Effect of Cr(VI) ions interaction with paper, water, carbohydrazide (CHD), and g-C<sub>3</sub>N<sub>4</sub> under UV light; Figure S21: Effect of interferences: g-C<sub>3</sub>N<sub>4</sub>-coated POCT kit interaction with water, carbohydrazide (CHD), Na<sup>+</sup>, K<sup>+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Fe<sup>2+</sup>, Cr(VI) ions, Hg<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Cr<sup>3+</sup>, and mixed cations (mcs).

Author Contributions: Conceptualization, M.M.; methodology, M.M.; software, M.M.; validation, M.M.; formal analysis, M.M.; investigation, M.M.; resources, M.M.; data curation, M.M. and N.Y.L.; writing—original draft preparation, M.M.; writing—review and editing, N.Y.L.; visualization, N.Y.L.; supervision, N.Y.L.; project administration, N.Y.L.; funding acquisition, N.Y.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIT) (RS-2023-00208684) and also by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R1A6A1A03038996).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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