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Graphene Oxide Derived Carbonaceous Sensor: Turn-off Fluorescence Sensor for Nanomolar Detection of Ruthenium ion in Aqueous Medium

Jeyaraj Belinda Asha, Murugan Karthik, Palaniswamy Suresh

Ruthenium, a vital transition metal widely employed in diverse applications from medicinal to material applications. Though ruthenium has positioned as safe and relatively less toxic than other metals, continuous exposure will cause undesirable effects. Due to the constant increasing usage of ruthenium, a simple and straightforward detection methodology is immediately necessary. In the present study, a covalently functionalized graphene oxide derived chemosensor has been developed to detect Ru(III) under a complete aqueous medium. For this, graphene oxide (GO) is covalently functionalized on edge carboxylic acid group with 2-(bis(pyridine-2-ylmethyl)amino)ethan-1-ol (PAE) through esterification and followed by further grafting on the basal plane with dansyl fluorophore motif through silyloxy propyl linkage. Each functionalization has designed to create a feasible pathway for Förster Resonance Energy Transfer (FRET) donor and acceptor energy transfer, where the metal-binding site is introduced through esterification and signalling unit by dansylation process. When exploring the application of newly prepared GOPND carbonaceous material, a selective fluorescence quenching response has been observed with Ru(III) in an aqueous medium which is utilized for the detection of Ru(III). GOPND probe shown high selectivity towards Ru(III) over the presence of other common metal ions with a lower detection limit of 15 nM. The origin of this 'turn-off' sensing response explained through the presence of both bis(pyridyl) probe and the dansyl fluorophore in close proximity on GO, which facilitates the energy transfer. The specific turn-off fluorescence response of the GOPND with Ru(III) ion is rationalized through FRET mechanism.

Introduction:
Graphene oxide (GO) a unique and versatile carbonaceous material in the contemporary materials research field. GO has attracted massive attention in developing novel materials for real-time application due to its inherent and exceptional physical, chemical, electronic, and optical properties. Though graphene and graphene oxide (GO) have a growing demand in material-based applications, the graphene research fields’ most exciting aspects are developing functionalized graphene materials with organic and inorganic motifs to enhance or fine-tune their functional properties. Due to the remarkable optical and electronic properties of the covalently modified GO, it has received enormous attention in optical and sensor applications. Though several physisorbed sensor probes were reported in the literature, the poor signal reversibility, and reproducibility, leaching of the physisorbed probes, and loss of active sites have indulged the development of a reliable sensor system. False-positive signals were detected in many cases of physisorbed or non-covalent functionalized GO sensors, which strongly insist on the need for covalent functionalization. Notably, interaction with other molecules or ions, optical and luminescent behaviours have significantly improved through covalent functionalization. The intrinsic electronic property of GO yields a special characteristic feature of the energy acceptor in the energy transfer process. In GO-based sensors, the fluorescence resonance energy transfer process takes place predominantly, and GO acts as an acceptor for efficient quenching of nearby fluorescent or through the covalently attached species owing to the sp² domains.

Though the photophysical nature of the GO widely studied as a quencher, however, under certain environment and presence of other external stimulus, the luminescent nature of GO was enhanced. Such increment in the PL behavior of GO and its physisorbed or chemically modified derivatives have been used for diverse sensing applications. In recent day, the chemosensor application of GO derived materials has continuously explored in the detection of heavy metal ions and even for f-block elements, organophosphorus pesticides, ATP and GTP monitoring in living cells. Maximum reports focused on the quenching of the PL through either blocking or unblocking of the graphitic surface of the GO through physisorbed molecules over the surface or decorated nanoparticles. Recently, the detection and removal of chromium(IV) ions proposed using graphene-based system. In the GO-based sensors, the non-covalently modified shown excellent response, indeed, none of the studies disclosed their stability and reproducibility for further application, which strongly questions the reliability of such sensor materials. Lack of adequate studies on the stability of the non-covalently modified GO-based sensors. With this ambiguity, in the present work, we have designed and developed a turn-off fluorescence sensor through covalent modification of both the edge carboxyl group and the basal

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*a* Supramolecular and Catalysis Lab, Department of Natural Products Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai-625021, India.

**b** Dept. of Chemistry, National Institute of Technology, Tiruchirappalli

*Corresponding Author. E-mail: *suresh.chem@mkuniversity.ac.in, gchemistry@gmail.com; Tel.: +91 9790296673.

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plane of the GO. A turn-off sensor material has been designed and developed based on the fluorescence resonance energy transfer between the coordinated metal site and luminescent probe attached on GO, where an organic motif 2-bis(pyridine-2-ylmethyl)amino)ethan-1-ol (PAE) used as a metal coordinating site, which covalently attached to GO through Steglich esterification and a dansyl motif as a fluorescence probe grafted on the basal plane through a propyl silyloxy linkage. This sensing material is designed to enhance benignness and also to detect ruthenium metal in an aqueous medium. With such a perspective, GOPND has been designed with FRET based sensing mechanism.

Ruthenium is a rare and heavy transition metal has diverse attractive application from medicine to material application. Ruthenium has found a potential alternative to platinum in medicinal application and is used as an immunosuppressant and dental alloy, anti-Alzheimer’s agent in microbial antibiotic production for Malaria, Chagas’s disease, septic shocks, and cancer treatment. Ruthenium derived drugs are gaining more attention in clinical application owing to their low toxicity. Meantime, ruthenium is an inevitable candidate in electronics due to excellent bandgap energies; it is gaining more attention in clinical application owing to their use in medicine to material application.

On another side, ruthenium has an inevitable role in synthetic organic chemistry as a potential catalyst. After the 2005 Nobel prize for olefine metathesis to form C-C bond, the demand for ruthenium derived catalyst has increased exponentially to synthesize active pharmaceutical ingredients. Besides, reactions such as that Fischer-Tropsch and Boulton-Katritzky also utilizes the ruthenium as a catalyst. In asymmetric synthesis, ruthenium-derived chiral catalysts are employed to fine-tune the enantioselectivity. However, it is challenging to remove and quantify the deeply colored ruthenium by-products from the desired product. Though ruthenium found numerous applications and has a pivotal role in human life’s welfare, its fate after usage is not determined correctly. Indeed, exposure, extensive use, and improper disposal of the ruthenium ions could also cause adverse effects in the environment, which exhibits various forms of toxicity and leads to health issues like platinum group elements (PGEs). The maximum permissible intake of Ru(III) or related transition metals in oral dosage and parenteral dosage in pharmaceutical products per day are 10μg/g and 1.0μg/g respectively. Apart from its extensive applications, in general, most of the ruthenium-containing compounds are considered as toxic because they can stain skin strongly, and ingested metal may retain strongly in the bone. Excess exposure or intake of the ruthenium is harmful and may cause corrosion to the respiratory tract, eyes, and digestive tract. The ruthenium’s toxic nature is also noted quite seriously since it has a mutagenic effect in normal cells. Due to the more extended stay of ruthenium complex on lungs affects the lung metastatic resulted in metastatic tumours. To detect the ruthenium ion’s existence, routine analytical techniques such as atomic absorption (AAS) and inductively coupled plasma-mass spectrometry (ICP-MS) are used. However, they involve expensive instrumental analysis and a rigorous sample preparation method. To the best of our knowledge, there is no study on the development of a chemosensor or detection of ruthenium except a few reports. A colorimetric methodology was reported using per-6-amino-β-cyclodextrin as a supramolecular host to detect Ru(III). Few of the fluorescence detectors such as porphyrin macrocyclic, fluorescein derivative through the oxidative process, CdTe quantum dots and 3D luminescent indium-organic framework and functionalized phenanthroline derivatives were used to detect Ru(III) along with aggregation-induced emission-based probe for Ru(III) also reported. Though numerous metal detecting methodologies and chemosensors have continuously evolved for several common metal ions, however, for the biologically and industrially essential metal ruthenium, very few sensing methods were developed. Indeed, a suitable and selective detection methodology is an immediate need. A reliable and straightforward sensing technique must be designed to detect such pharmaceutically cum industrially versatile metal ions from the environment and biological system.

We have developed a “turn-off” fluorescence sensing strategy for the rapid and selective detection of ruthenium(III) in an aqueous medium. Based on hard acid and soft base (HSAB) theory, bis(pyridine) motif as a metal coordination site, and dansyl motifs attached with GO as fluorescent probe, a heterogeneous fluorescence sensing probe is designed to operate through FRET sensing mechanism. With this fundamental designing, when ruthenium(III) coordinates with GOPND and subsequent excitation, an excited state energy transfer takes place through FRET from ruthenium(III) coordinated bis(pyridyl) to dansyl attached on the basal plane of GO, a simple turn-off type fluorescence sensing process would take place; also the calculated LOD is relatively less than previously reported. Furthermore, unlike the homogenous chemosensors, the heterogeneous chemosensors will strongly support scaling up and to design the materials for real-time sensor applications.

Experimental Section

Materials and Methods

2-Aminoethanol (Sd Fine-Chem. Ltd.), 2-(chloromethyl)pyridine hydrochloride, and 4-dimethylaminopyridine, N, N'-dicyclohexylcarbodiimide (Spectrochem) were used as received. Pristine graphite (Sigma-Aldrich), NaNO₃, potassiumpermanganate, sulfuric acid, hydrochloric acid, potassiumcarbonate, AgNO₃, Al(NO₃)₃·9H₂O, BiCl₃, CaCl₂, CoCl₂, CuSO₄·5H₂O, Cr₂(SO₄)₃, FeCl₃, HgSO₄, KCl, La₂(SO₄)₃, Mg(NO₃)₂, NaCl, Ni(NO₃)₂·6H₂O, Pd(NO₃)₄, Rh₂(C₂H₅COO)₆, RuCl₃, SnCl₂, ZnCl₂ and ZrCl₄ (Merck), triethylamine, (3-amino propyl)triethoxysilane, and dansyl chloride (Alfa Aesar) were used as received without further purification.

Characterization

Elemental composition was (CHN) examined using Perkin Elmer USA, 2400 series 2. Fourier-transform infrared spectroscopy (FTIR) spectra were documented in a Shimadzu instrument from 4000 cm⁻¹ to 500 cm⁻¹, using the KBr pellet technique. FT-Raman spectra were studied in Shimadzu

Materials Advances
Page 2 of 11
instrument with laser 630 nm. Powder X-ray diffraction (PXRD) analyzed (XPERT-PRO) using Cu Ka radiation at room temperature. Scanning Electron Microscopy (SEM) analysis was performed in a TESCAN VEGA3 instrument using the SE detector and furnished with an Energy Dispersive Analysis of X-rays (EDX). Samples for High-Resolution Transmission Electron Microscopy (HR-TEM) images obtained from FEI-TECNAI G2 200 KV TEM instrument. X-ray photoelectron spectroscopy (XPS) was recorded using Omicron Nanotechnology spectrometer (hν = 1486.6 eV). 1H NMR and 13C NMR spectra were analyzed using Bruker spectrometer of 300 MHz and 75 MHz, respectively. Electrospray ionization mass spectrometry (ESI-MS) analysis was performed in an LCQ Fleet-Thermo Fisher instruments limited, USA. For the absorption and emission spectral studies, the aqueous solution of GOPND, and all-metal solutions were prepared using double distilled water and maintained the pH 7 using PBS (Phosphate-buffered saline) buffer. UV-vis. spectra were recorded in Agilent diode array 8453 spectrophotometer. Photoluminescence (PL) emission spectral studies, the aqueous solution of GOPND, and all-metal solutions were characterized using various analytical techniques.

Results and Discussion

In the present work, a carbonaceous sensor was developed from graphene oxide (GO) through covalent functionalization with energy donor and acceptor to detect Ru(III) using the fluorescence technique in an aqueous medium. Scheme 1 First, the 2-bis(pyridine-2-ylmethyl)amino)ethylgraphenate (GOP) is prepared from GO through Steglich esterification with PAE. The graphitic plane of the GOP covalently grafted with silyloxy aminopropyl using APTES, and subsequently, the primary amino group present on resulting GOPN was reacted with dansyl chloride, finally gave the probe GOPND. In the GOPND, the bis(pyridyl) motif and the dansyl probe are covalently attached with the edge carboxylic group and on the basal plane of GO, respectively. Clearly explains about the preparation process in a step-wise. The as-prepared GOPND sensor material and its corresponding precursors were subjected to systematic characterization using various spectroscopic and microscopic analysis at each stage of preparation. All characterization precisely revealed the unique functionalization. The preliminary elemental analysis results of GOP, GOPN, and GOPND showed a notable increase in the elemental composition (fig. 1 (i)). A significant enhancement in the element ratio and 12 % of nitrogen supports the formation of GOPND.
Next, the covalent functionalization was characterized using Fourier transform infrared (FT-IR) spectroscopy analysis; it is an elegant tool to analyze graphitic material’s chemical functionalization. The complete covalent modification and formation of GOPND is confirmed through the meticulous analysis of FT-IR spectra and comparison with its precursors (fig. 1(iii)). The presence of a peak at 1722 cm\(^{-1}\) representing the C=O stretching of the ester linkage and indicated the functionalization of bis(pyridyl) motif on edge carboxylic groups of GO. Added to that, the stretching at 1575 cm\(^{-1}\) of the aromatic C=C stretching shows the presence of pyridyl rings of bis(pyridyl) motif. Compared to GO, new peaks that appeared in the range of 1032 cm\(^{-1}\) to 1122 cm\(^{-1}\) correspond to the Si-O and Si-O-C stretching frequencies confirmed the functionalization of aminopropyl on the basal plane of the GO through silyloxy linkage. Further, the peak at 3377 cm\(^{-1}\) of the primary amine in GOPN shifted to 3405 cm\(^{-1}\) in GOPND corresponds to the stretching frequency of secondary amine, demonstrates the functionalization of dansyl chloride. Further peaks at 1390 cm\(^{-1}\) of sulfonil, 2491 cm\(^{-1}\) of S=O and N-H stretching frequencies, and 1473 cm\(^{-1}\) of symmetric bending stretching frequency of methyl group support the functionalization of dansyl motifs with GOPN. The peaks at 785 cm\(^{-1}\) and 1520 cm\(^{-1}\) of naphthalene stretching frequencies also strongly ascertain the presence of the dansyl motif in the covalently functionalized GOPND. The peaks 2669 cm\(^{-1}\) and 2928 cm\(^{-1}\) of alkyl stretching frequencies show the propyl linkage between the dansyl motif and the graphitic plane of GOPND. Furthermore, the peaks at 1645 cm\(^{-1}\) and 1210 cm\(^{-1}\) of aromatic C=C and C-C ascertain the presence of the graphitic plane.

![Fig. 1](https://example.com/fig1.jpg)

Fig. 1 Spectroscopic analysis of GO, GOP, GOPN, and GOPND. (i) Elemental analysis, (ii) FTIR, (iii) Raman spectra, (iv) Powder X-ray diffraction (PXRD) pattern.

material, such as the degree of functionalization and defects derived from the functionalization. The as-prepared all the materials GOP, GOPN, and GOPND, were analyzed in FT-Raman, and the results are presented in fig.1(iii). From the Raman spectra, the existence of sp\(^2\) and sp\(^3\) hybridization, the corresponding change in the D/G ratio of derivatized GO materials reveal functionalization. For GO and GOP, the calculated D/G ratios are 0.83 and 0.84, respectively, and I\(_D\)/I\(_G\) are 1 and 1.01, respectively, which indicates that no notable change occurs on the GO basal plane. Similar results were continued on further functionalized GOPN and GOPND. The D/G ratio of GOPN and GOPND are 0.83 and 0.84, respectively and I\(_D\)/I\(_G\) ratios are 1.03 and 1.02, respectively. The analysis of both D/G and I\(_D\)/I\(_G\) ratios revealed no significant changes apart from a marginal increase in the peak intensities. It discloses that the esterification and the silylation on the basal plane of the GO did not affect the hybridization of the carbon on the graphitic domain because the silyloxy propyl functionalization takes place on the existing hydroxyl and epoxide functional groups. The FT-IR analysis also supports this observation through the unaffected C=C peaks at 1626 cm\(^{-1}\).

Further, to verify the crystalline nature of the as-prepared GOP, GOPN, and GOPND, they were subjected to powder X-ray diffraction (PXRD) analysis. However, the PXRD pattern does not provide any remarkable information regarding the functionalization and formation of GOPND material. In general, GO shows two characteristic broad peaks at a 2θ value of 12.2° and 41.6° corresponding to (001)\(^{20}\) and (100)\(^{21}\) reflection planes, respectively. In the case of GOPND after the covalent modification, the same two planes are retained, whereas the 001 planes slightly shifted to 12.4°. However, compared to GO and GOP, peaks get broader, which affirms that the amorphous nature has maintained even after functionalized with silyloxy bonding. Indeed, the interlayer spacing of GO could be tuned through the covalent functionalization. The calculation of the interlayer spacing of GOPND using the Bragg’s equation showed an intriguing enhancement in interlayer spacing of 2.3 nm from 0.725 nm with a slight decrease of 2θ (001) = 12.779 to 12.43, which ascertained the covalent functionalization of GO and resulted from the formation of GOPND. Though all the above characterization gave an understanding regarding the formation of GOPND from GO, finally, to unambiguously understand the covalent functionalization and confirm the formation of GOPND, X-ray photoelectron spectroscopy studies (XPS) were carried out. While analyzing the XPS spectrum of the GO, only two significant peaks of C 1s (286.4 eV) and O 1s (533 eV) are present. After covalently modified, in the XPS spectrum of GOPND, in addition to the enhanced intensity of C 1s and O 1s, new additional peaks N 1s, S 2p, and Si 2p have appeared.

The deconvoluted C 1s spectrum at 284 eV of GOPND gives three peaks 283.6 eV of sp\(^2\) of C-C\(^{22}\) of the graphitic basal plane, 284.7 eV\(^{23}\) of aromatic C from benzene and naphthyl rings, and 286.7 eV of C-O from the ester carboxylate.\(^{24}\) The peak at 531.3 eV corresponds to O 1s, which validates the presence of ester carboxyl (C=O).\(^{25}\) Next, when deconvoluting the peak N 1s at 399 eV, give two peaks at 398.9 eV and 401 eV of pyridinic nitrogen of bis(pyridyl) unit and ‘C-N’ of N, N-dimethyl unit from the dansyl motif, respectively. These nitrogen peaks confirmed the covalent attachment of the bis(pyridyl) and dansyl group with the GO. Further, the presence of two peaks at 231 eV and 167.6 eV corresponds to the sulfur 2s and 2p binding energy present as a sulfone group in the dansyl motif. Finally, the peak 101.5 eV proves the
silyloxy functionalization on the GO basal plane and further deconvolutes give two peaks 101.2 eV of Si-O/Si-O-C and 101.9 eV of Si-C. The above-detailed observation from the XPS survey strongly confirms the formation of GOPND.

SEM images usually demonstrate a sheet-like morphology for GO; here, the same has been observed, but in the case of GOP (Figure 3(b)), certain voids are detected due to the covalent modification. The SEM images show a crushed and pulverized form, and it reveals that the functionalization may introduce a lot of defects over the surfaces; the sheets were not uniform, there are a lot of pits found on the surface due to its surface defects. Silylation introduces a flake-like morphology followed by a network like morphology for fluorophoric modification. Fig. S4 of supporting information gives the elemental mapping of GOPND; also, the elemental percentage endorses the result of CHN analysis. HR-TEM images (Figure 3 e-i) denotes the same as that of SEM; the functionalized GO also formed in sheet-like morphology with multiple stacking of layers in all the steps, the presence of wrinkles on the sheets notified the functionalization. The functionalized GOPND finally exists as a clear sheet-like morphology without any crumbling or flooding, which agrees with the SEM images.

After meticulously characterized the as prepared GOPND material, its application has been disclosed as a heterogeneous carbon derived sensing probe to detect environmentally influential analytes. The presence of the bis(pyridyl) motif in GOPND is expected as an analyte binding site, the luminescent dansyl motif as a signalling unit, and both are synchronized together and anticipated to give good PL response through energy transfer pathway. The as-prepared GOPND has good dispersibility in water, which gives an excellent UV-vis spectrum with three absorption bands at 214, 268, and 369 nm. It’s respective precursor GOP showed absorption at 265 and 287 nm, and successive silylated GOPN gave peaks 229 and 258 nm. When compare to electronic spectra (Figure S5) of all precursors and free organic motifs bis(pyridyl) and dansyl chloride, after the functionalization, the three peaks of GOPND were got red-shifted to longer wavelength owing to the energy transfer capability of the GO; it further evident the covalent functionalization. In the solubility studies of GOPND with different solvents, a stable dispersion was observed in water than other organic solvents; hence all absorption and emission studies were carried out in an aqueous phosphate-buffered solution.

From all the above spectroscopic and microscopic analyses, the covalent modification was unambiguously characterized and based on the characterization results, a plausible structure of GOPND is proposed (Scheme 1).
saline (PBS) buffer medium maintaining the pH 7. In general, dansyl chloride is insoluble in water; however, the covalently functionalized GOPND offers a better dispersibility also enhances the solubility of dansyl, further resulted an excellent green, fluorescent when exited at 365 nm. Fig. S6 revealed the emission spectra of PAE, GOP, GOPN, DCL and GOPND at $\lambda_{ex}=370$ nm. Further to understand the absorption response of the GOPND with a series of metal ions including alkali, alkaline earth, p-block, transition, and rare earth metals with different oxidation states such as Ag(I), Al(III), Ca(II), Co(II), Cr(III), Cu(II), Fe(III), Hg(II), K(I), La(III), Mg(II), Na(I), Ni(II), Pd(II), Rh(II), Ru(III), Sn(II), Zn(II) and Zr(IV). But all the metal ions did not show any distinct response with the GOPND probe, and no change in the absorption intensity; however, a blue-shifted was noted.

Further, to explore the interaction of metal ions with the probe, an absorption titration was carried out using GOPND (10 ppm) with a random concentrations of metal ions (10, 100, and 200 ppm) in which except Ru(III) all-metal ions did not show any response (Fig. S7). Whereas after the addition of 100 ppm of Ru(III), a visual enhancement was found with a blue-shifted absorption band at 281 nm, and it continued with 150 ppm. With these encouraging results, a complete titration using 10-200 ppm of Ru(III) was carried out, and an apparent enhancement of the blue-shifted bands at 281 nm was observed, and further, it gets shifted to 276 nm. The change in the absorption is rationalized by the coordination of Ru(III) with the bis(pyridyl), which arrest the energy transfer to the dansyl motif in the ground state, and it is also revealed by the absence of no visual colour change. The preliminary absorption studies showed a notable change while interacting with Ru(III). However, it does not provide any informative results regarding the sensing of Ru(III) ion, to develop a reliable sensing methodology further moved to fluorescence studies. The PL studies of the GOPND were carried out in an aqueous PBS buffer. Here it is essential to remember that GO and its derived materials do not obey Kasha’s rule\(^{78}\) in polar solvents; it exhibits a giant red-edge shift. To find the best excitation wavelength for GOPND in the polar PBS medium, the screened wavelengths 360, 370, and 380 nm (10 ppm), at 370 nm showed an excellent PL response in 519 nm with a maximum intensity in minimum concentration (Fig. S8).

Under the similar excitation wavelength except for dansyl chloride, other precursors PAE, GOP, and GOPN are non-emissive (Fig. S6). The PL intensity of GOPND was slightly decreased than the free dansyl chloride under the identical condition owing to the quenching efficiency of GO. With the above-screened excitation wavelength, all-metal ions tested in absorption studies such as Ag(I), Al(III), Ca(II), Co(II), Cr(III), Cu(II), Fe(III), Hg(II), K(I), La(III), Mg(II), Na(I), Ni(II), Pd(II), Rh(II), Ru(III), Sn(II), Zn(II) and Zr(IV) were excited with GOPND. Not like absorption results, the PL studies shown a distinct response, where all the tested metal ions were highly inert without any influence on the PL spectra of GOPND (fig. S9) except Ru(III).

When treating 10 ppm of Ru(III) with 10 ppm of GOPND showed an immediate fluorescence quenching when excited at 370 nm. Even with higher concentrations (10 ppm, 100 ppm and 200 ppm), none of the other metals quenches the fluorescence enhancement of the GOPND (figure S9).
It reveals that all metal ions other than Ru(III) do not have any remarkable riposte towards GOPND. The images of GOPND under UV lamp in the presence of different metal cations is a visual proof of selective fluorescence quenching for Ru(III). It reveals the response of Ru(III) cation towards GOPND, all the other cations does not show any quenching rather it slightly enhances the fluorescence than Ru(III). Ru(III) selectively quenches the PL intensity. Even the increased concentration of metal ions does not vary the intensity as like Ru(III) which shows seldom response whereas the decreasing and increasing concentration of Ru(III) gives respective PL spectra as well as its image format too. Further, to explore the sensing behaviours of GOPND, a titration was carried out. During the sequential addition of Ru(III) with an increasing 10 ppm scale, a complete quenching was noted after the addition of 200 ppm. As observed in figure 8, an immediate quenching in the PL response of the GOPND in the presence of Ru(III) at $\lambda_{ex}$=370 nm strongly represents the high selectivity of the GOPND towards the detection of Ru(III) in the aqueous medium.

Though the GOPND showed an excellent selectivity by decreasing its PL intensity with Ru(III), the selectivity, sensitivity, and the competitive sensing potential of the probe in the presence of the other common metal ions were also investigated, and the results are presented in figure 9. Among all the screened metal ions, Ru(III) ion's quenching response with GOPND was unique and incomparably higher. During the competitive studies between Ru(III) with other metal ions, on the addition of different metal ions even with a higher concentration of 50 ppm together with 50 ppm of Ru(III), not shown any notable change in the quenching behavior; indeed, the actual PL quenching nature of the Ru(III) with GOPND was retained up to 2%.

The same response was retained even after raised the other metal ion concentration to 110 ppm. Meanwhile, some of the other metal ions meagrely influenced the Ru(III)'s quenching efficiency. Metal ions such as Cu(II) and Rh(II) ions quenched the PL intensity around 19-20% along with Ru(III) ions; however, this behaviour was not shown when those ions individually titrated. Nevertheless, the competitive quenching behaviour of other metal ions does not influence Ru(III)'s selectivity with GOPND. The competitive studies unambiguously demonstrate that the GOPND behaves as an efficient and specific sensing probe for the Ru(III) ions, even in the presence of other metal ions with excess concentration.

In the development of the materials as a sensing probes, the pH of the medium is highly inevitable. It is one of the decisive parameters to fine-tune the sensing potential of the developed sensor probe. The as-prepared GOPND has several nitrogen centers in $\text{bis}$(pyridyl) and dansyl motifs; thus, understanding the role of pH in the sensing medium is essential. The sensing behaviours of the GOPND probe with Ru(III) in different pH ranges from 1-14 were studied by tuning the buffer in an aqueous medium. The observed PL quenching response of the GOPND in the presence of Ru(III) under different pH ranges has been presented in figure 10. The analysis of the pH response plot (Figure 10) disclosed the change in the pH candidly influences the probe’s sensing response. When decreasing the pH towards the acidic, the quenching process starts falling. At pH 5, 6 and 7, probe not much influenced, only 8 to 18 % of the decrease in the quenching efficiency was noted. However, after pH 4, a sharp
reduction of around 68 to 83% in the probe’s quenching efficiency was observed. This behavior is rationalized through the protonation of the nitrogens present on GOPND and blocked the binding sites of Ru(III). On the other side, a similar trend was repeated while increasing the pH to 8, and a similar decrease in the quenching efficiency was observed up to pH 11. After pH 12, a sudden drop in the quenching behavior of the GOPND was observed and only 13% of quenching was noted to the higher pH 14. This quenching studies of the probe under different pH conditions and change its efficiency strongly endorse that the prepared GOPND probe’s sensing behaviour have been influenced by pH and only under the physiological neutral pH is more responsive.

Besides, the detection ability of the GOPND probe was also monitored using the same stock solution prepared before 48 h, and a negligible change in the sensing behavior was observed. Both the studies strongly endorse the stability of GOPND probe in the aqueous medium in Ru(III) detection, which strongly helpful to use this sensor probe for periodical applications.

Unlike homogeneous chemosensors, probe derived from carbon-based materials are highly stable and retained their PL capability for a long time without suffering any chemical and physical changes. The present GOPND probe is graphene derived material and heterogeneous in nature. Hence to understand the stability and PL response of the prepared GOPND probe in PBS buffer media, fluorescence emission intensity and peak shapes are monitored concerning time. When periodically checking the probe alone fluorescence intensity in 12 h interval revealed that the GOPND is stable in PBS buffer even after 48 h without any remarkable change in peak shape and emission intensity.

Next, to understand the necessity and significance of the covalent modifications on the GO basal plane in the present Ru(III) sensor study, control experiments were pursued using the corresponding precursors used to prepare GOPND. A separate PL study of GO, PAE, and dansyl chloride showed that none of them interact with Ru(III) even at high concentration (200 ppm) and showed no response except GOPND and Ru(III) (10 ppm) (Fig. S10). Further, another successive precursor, GOP, in which the bis(pyridyl) attached with GO through ester bond, is highly inert towards Ru(III) without expelling any PL response when excited at 370 nm. Meantime, the same GOP probe is highly responsive towards Zr(IV) and explored as a turn-on fluorescence sensor. A similar inert response was observed with GOPN also (Fig. S10).

All above control studies clearly confirmed that complete functionalization of GO with bis(pyridyl) and dansyl motif and the resulting covalently modified GOPND could only act as a responsive turn-off sensor for Ru(III). In another study, the physical mixture of GO (2.5 ppm), PAE (2.5 ppm), APTES (2.5 ppm), and dansyl chloride (2.5 ppm) along with Ru(III) (100 ppm) were tested and the physical mixture did not show any fluorescence quenching.

It reveals that the physical mixture does not exhibit the fluorescence as that of the GOPND further the quenching behavior not originated owing to the non-covalent binding or the physisorption (Fig. S11). In another study, to understand the fate of the GOPND after the Ru(III) sensing, the used probe was collected and recovered by centrifugation. The recovered probe was repeatedly washed with water and dialyzed three times in DD to remove the Ru(III) ions and finally washed with dry acetone. The complete removal of ruthenium ions and the absence of other species, if any, in the recovered GOPND was confirmed by the absorption and emission analysis of filtrate. Additionally, it was dried under vacuum for 24 h and subjected
to FT-IR analysis. The FT-IR study demonstrates that the GOPND probe gets disturbed after the sensing process and the dansyl motif cleaved from GOPND. The absence of the peaks at 785, 1210, 1473, 1390, and 2491 cm⁻¹ correspond to the dansyl motif revealed the probe’s cleavage, and the FT-IR of the recovered samples agreed with GOPN. So, after the sensing process completed, the recovery process may disrupt the sulfonyl amide bonding of GOPND, albeit the rest of functionalization (GOPN) not affected (fig. S12). To examine the application of the developed GOPND probe in real-time applications, further studies were carried with the samples collected from natural sources, aqueous workup of ruthenium-catalyzed reaction, and laboratory effluent. In synthetic organic chemistry and catalysis research, the ruthenium-based catalyst has widely been consumed. Notably, ruthenium-derived homogeneous catalysts has gained wide application, and after reaction completion, they were discarded as a waste. To detect ruthenium from such effluent, we were chosen three samples to examine the sensing potential of the GOPND. The aqueous work up from RuCl₃ derived asymmetric catalysis reaction, and another sample collected from the academic research laboratory drain out. One more water sample was collected from the Vaigai riverbeds of India. The collected samples were filtered through a 2µ filter, and the resulting clear aqueous solution was only taken for analysis. For the real sample analysis, a 1:1 ratio of GOPND and samples were taken under the optimized identical condition and excited at λₑx = 370 nm. Both the samples collected from laboratories showed an impressive response and a significant quenching in the PL intensity of GOPND. Meantime a weak response was inferred from the river water samples. The comparative study of the real-time samples with standard titration measurement shows the presence of 110, 100, and 20 ppm of Ru(III) from aqueous workup, laboratory drain, and river water samples, respectively.

The response and results from the real-time samples strongly ascertained the GOPND probe’s efficiency in the detection of Ru(III). The Stern-Volmer plot explains about the quenching constant i.e., it’s more significant for Ru(III) since Kᵥ=9.9 × 10⁸ M⁻¹ (figure. S13). The LOD value calculated from Stern-Volmer plot using the standard formula (3σ/k). Where ‘o’ is the concentration of Ru(III) and ‘k’ is slope obtained from the plot of Stern-Volmer. Using the above equation, the LOD of the GOPND probe calculated as 15 nM. The sensing ability of the GOPND towards Ru(III) through fluorescence quenching is rationalized by proposing a mechanism based on the literature reports. In general, GO exhibits excellent emission properties owing to the presence of an extended conjugation system and diverse oxygen functionalities, however, in most of the PL studies, due to the charge transfer process, the fluorescence response was quenched by various mechanistic pathways and positioned as a turn off sensor. With a similar strategy, the present highly fluorescent GOPND covalently functionalized with bis(pyridine) and dansyl motifs acting as a turn-off sensor when selectively binds with Ru(III). Here, the GO basal plane provides a better platform for electron-hole recombination, which is induced by the electronic transitions between the non-oxidized carbon atom regions and oxidized carbon atom regions. The prepared GOPND probe was designed based on the energy transfer phenomenon, where the energy acceptor and donor are separated by the Förster energy distance. In which the silyloxy propyl unit acting as a spacer between bis(pyridine) functionalized GO and dansyl motif, it all comes under close proximity. From the literature, it is understood that the dansyl motif acting as an energy donor, and the bis(pyridine) unit is positioned as an acceptor in GOPND. GOPND itself emits green fluorescence at 365 nm in an aqueous medium owing to the presence of the dansyl motif.

While exciting at 370 nm, merely at the excitation wavelength of dansyl chloride gives maximum fluorescence enhancement in an aqueous medium which is due to the giant red edge effect of the GO. (it possible only due to the functionalization of dansyl with GO, hence the dansyl motif alone has low or no fluorescence nature under an aqueous medium). Meanwhile, the addition of the Ru(III) readily and selectively binds with the bis(pyridine) owing to the existence of a pseudo cavity by two pyridinic nitrogens and one aliphatic tertiary nitrogen (hard base). When exciting the GOPND, after the addition of Ru(III), a synchronized energy transfer from the dansyl motifs to the Ru(III) bound bis(pyridine), would take place through Förster resonance energy transfer. After the energy transfer from the dansyl unit to the Ru(III) bound bis(pyridine), lack of sufficient energy required for the free rotation of the N, N-dimethyl group of dansyl motif and the restricted conjugation of lone pair of electron decrease the fluorescence intensity and resulted in quenching. The same quenching behavior could also be possible by other quenching mechanistic pathways like ICT and PET; however, albeit the energy donor dansyl and acceptor bis(pyridyl) are present in the same GO domine as a single molecule, owing to the lack of extended conjugation, other energy transfer mechanism is not
possible. The same behavior is continued when increasing the concentration of the Ru(III) and the PL of GOPND was completely quenched after the addition of 200 ppm of Ru(III). On the other hand, in order to understand the role of the covalently functionalized GO in the present Ru(III) detection process, a controlled study was carried with a 1:1:1 physical mixture of the GO, 2-bis(pyridine-2-ylmethyl) amino)ethan-1-ol and dansyl chloride, which did not show any fluorescence quenching response under identical studied condition (Fig. S11). This control study strongly ascertained the importance of the covalently functionalized GOPND, where the bis(pyridine) and dansyl motif are brought under proximity on the graphitic dome and facilitate the FRET-based energy transfer to detect Ru(III) in the aqueous medium. Further the efficiency and sensitivity of the present GOPND probe is compared with the previously reported Ru(III) detection methodologies (Table S1). According to the table S1, so far reported probes shown LOD in the region of micro molar concentrations and very few reports have the nanomolar region also the sustainability is still questionable and failed to explain the sensing mechanism. Here GOPND has shown reliable result with good sensitivity in nanomolar detection range and has more advantageous than other probes. The present carbonaceous sensor material has LOD of 15 nM in complete aqueous medium.

Conclusion
Novel and carbonaceous sensor material GOPND was synthesized through covalently functionalizing the pristine graphene oxide with PAE through Steglich esterification on the edges and dansyl motif on the basal plane. Three step functionalization promisingly provides a better PL response with Ru(III). The synthesized GOPND was thoroughly characterized using various possible and feasible analytical techniques which confirmed the covalent functionalization. The enhanced PL properties of the synthesized GOPND material in the aqueous medium has been used as a turn-off chemosensor for the detection of industrially and academically essential ruthenium(III) ions under the physiological pH with the lowest detection limit of 15 nM (31 ng/mL or 31 ppb). The efficient fluorescence quenching response of the GOPND unveils the high selectivity towards the Ru(III) even in the presence of other common metal ions. The selective sensing behavior of the GOPND towards the Ru(III) is explained through the FRET mechanism owing to the presence of both bis(pyridyl) and dansyl motif in proximity through covalent functionalization of GO. The real-time application of the GOPND proved by detecting Ru(III) from laboratory effluent and river water. The development of such carbon materials-based sensing methodology and selective detection ability from covalently functionalized graphene materials find a wide application in developing carbonaceous and straightforward materials-based sensor system for electronics and chemical applications.

Statement of Contributions
Preparations of materials, material characterization, manuscript preparation and analysis of results, J. B. Asha.; Formal analysis, assisting for the preparation of Materials, M. Karthik.; Conceptualization, investigation, manuscript preparation and supervision, Dr. P. Suresh.

Conflicts of interest
There are no conflicts to declare

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