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A TURN-ON DUAL FLUORESCENT SENSOR FOR CYSTEINE AND THIOUREA USING ACRIFLAVINE LOADED GRAPHENE OXIDE

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ABSTRACT

The existence of a dual sensor for the combination of cysteine and thiourea is very rare, despite the presence of numerous fluorescence sensors for cysteine in the literature. On the other hand, thiourea has been involved in fluorescence sensors for many metal ion sensors. Here, we have developed an inexpensive and rapid fluorescent sensor for this dual pair using acriflavine-loaded graphene oxide (GO–ACY) in water. Interestingly, GO–ACY displayed a good fluorescence for both cysteine and thiourea. Indeed, GO-ACY did not respond to other amino acids, excluding cysteine. Calculated LOD for thiourea was found to be 7.1×10^{-7} M, and for cysteine, it was found to be 8.4 nM. The presence of thiourea in pharmaceutical drugs also displayed a good fluorescence, indicating that the sensor could be useful for the estimation of drugs that have the thiourea moiety.

Keywords: Graphene oxide, Acriflavine, Drugs, Cysteine, Thiourea.

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INTRODUCTION

Cysteine and thiourea are sulfur-containing compounds that play important roles in chemical, biological, and pharmaceutical system.[1] Cysteine is an essential amino acid found in many proteins and is added to several medicines and nutritional supplements. Abnormal cysteine levels are associated with various health problems; therefore, the sensitive detection of cysteine is important in clinical and pharmaceutical analysis.[2] Thiourea, on the other hand, is commonly used in the pharmaceutical and chemical industries and can appear as an impurity in some drug formulations.[3] Unlike urea, high levels of the thiourea moiety are harmful; its accurate identification is important for drug quality control and environmental monitoring purposes. The existence of fluorescent sensors for the detection of cysteine is huge, which has been covered in recent review articles.[4,5] Besides, many other techniques also helped the detection of cysteine, including HPLC,[6] optical colourimetric detection,[7] capillary zone electrophoresis.[8] Furthermore, graphene oxide-based electrochemical sensors also exist for the detection of cysteine.[9] On the other hand, examples of fluorescence sensors for thiourea are limited. Electrochemical sensors,[10] colorimetric,[11] and Raman spectroscopy[12] methods are known for the detection of thiourea. Fluorescent dyes loaded on graphene oxide (GO)-based probes offer simple, fast, and selective methods for detecting many interesting compounds.[13] Fluorescence compounds such as carbon dots have been utilised for the sensing of analytes such as picric acid and metal ions.[14] On the other hand, GO binds non-covalently with aromatic dyes such as acriflavine (ACY), which turn off their fluorescence and subsequently, upon interaction with cysteine or thiourea, similar to the other GO-dye combinations, thiourea and cysteine also turn on their fluorescence due to the release of dyes from GO. We anticipate that the affinity of sulfur-containing compounds towards GO leads to the release of the dye, resulting in the release of the fluorescence compound that was loaded on GO.[15] This property allows the GO-dye supramolecular complex to act as a sensitive sensor for detecting cysteine and thiourea at very low concentrations.[13-17] There are a few reports for the sensing of cysteine that include the plasmonic nanoparticles that have been reported for the sensing of cysteine.[18] Cysteine is a key sulfur-containing amino acid, and copper nanoparticles have been

widely studied for their plasmonic properties, stability, and sensing applications. A recent review article compared cysteine-capped copper nanoparticles and copper-based sensors for cysteine detection, highlighting their synthesis, properties, and applications.[19] As the scarcity of fluorescence sensors exists for thiourea, we explored GO–ACY probe for its efficiency in detecting cysteine and thiourea in water and drug samples. Graphene oxide (GO) was first synthesised using a reported procedure, after which acriflavine (ACY) dye was assembled onto the GO surface through supramolecular interactions.[20-23] Our method of making the GO-ACY is cost-effective, and no synthetic skill is required as the GO and ACY are commercially available. Thus, our present work utilised the GO-ACY for the probing of thiourea and cysteine and a pharmaceutical drug, and the probe exhibited excellent sensitivity, with detection limits of 8.4×10^{-9} M for cysteine and 7.16×10^{-7} M for thiourea, indicating the efficiency of the sensor for various applications of biochemistry as well as the pharmaceutical industries. This study is aligned with SDG-3: Good Health and Well-being, as it focuses on the sensitive detection of biologically significant compounds such as cysteine, which are relevant to health and clinical analysis. The proposed sensing approach may support improved monitoring in pharmaceutical and biomedical applications.

EXPERIMENTAL

Material and Methods

All the chemicals, such as acriflavine, hydrazine hydrate, thiourea, cysteine, and other solvents used in this work, were analytical grade procured from AVRA and SRL, Otto Chemie, and Merck (India). GO was purchased from Amazon India and diluted appropriately. Besides a few of the pharmaceutical drugs, such as carbimazole, paracetamol, N-acetylcysteine, cetirizine, metformin, and thyroxine sodium were obtained from the local drug store. Fluorescence studies by Shimadzu RF-5301-PC spectrofluorophotometer and UV-Visible absorption studies were performed using a USB-2000 model spectrophotometer (Ocean Optics, USA).

Synthesis of Acriflavine Anchored Graphene Oxide (GO-ACY)

The GO-ACY material used in this work was prepared based on the reported procedure.[15,17] GO (0.1 g/mL, 4 mL) solution was mixed with acriflavine (1 mM prepared in 200 mL of water) and stirred until the GO dispersed completely. Subsequently, of 30% hydrazine hydrate (0.7 mL) was added dropwise to this mixture and stirred for 12 hours and kept undisturbed for 72 hours. The unbound acriflavine was removed by washing five times with water, followed by two washes with ether, and again three washes with water. After the last centrifugation, 10 mL of DI water was added, and the obtained material was used as a stock solution (GO-ACY) for detecting cysteine hydrochloride or thiourea.

Sensitivity of GO-ACY for Cysteine and Thiourea

Cysteine solutions were made in the range of 10^{-2} to 10^{-9} M, and Thiourea solutions were prepared from 10^{-2} to 10^{-7} M. Using the fluorescence response and the regression plots for Cysteine and Thiourea in the range 1×10^{-2} to 1×10^{-7} The limits of detection for both analytes were determined from fluorescence response and corresponding regression plots within the linear range of 1×10^{-2} to 1×10^{-7} M.

Selectivity of GO-ACY Towards Cysteine and Thiourea

The selectivity of GO-ACY, we have prepared solutions of glucose, amino acids, and several metal ions, at a concentration of 10^{-3} M in water.

RESULTS AND DISCUSSION

Sensitivity of GO-ACY Towards Cysteine and Thiourea

GO-ACY was prepared from GO and ACY as mentioned in the experimental section,[25-29] and utilised for the selective detection of both cysteine and thiourea. Thiourea and cysteine have been examined by the GO-ACY to understand the sensitivity of the probe towards the analytes. In the literature, we found no good probe is available to examine the presence of the thiourea moiety with good sensitivity and thus, we moved our interest in examining the GO-ACY for this purpose. Later, we found it worked for the cysteine along with thiourea. Thiourea is widely used in agriculture, industry, and analytical chemistry, but is known to be toxic and environmentally harmful. Existing detection methods for cysteine and thiourea often require complicated instruments and rigorous synthetic methods for the preparation of probe. However, the ACY-loaded GO helped to promote the sensing of thiourea and cysteine with exceptional

sensitivity and good selectivity. Furthermore, the preparation of the probe and testing of the probe can be carried out conveniently, and no special technique or difficult procedure is involved. For example, the presence of cysteine was examined with the concentrations ranging from 10^{-3} to 10^{-10} M solutions in different vials. From that, 2 mL of cysteine was taken, and to that, 50 µL of the GO-ACY probe was added, and the released dye was examined as an indirect concentration of cysteine. Similarly, thiourea was examined by the preparation of standard solutions from 10^{-3} to 10^{-6} M in water. To measure the concentration of thiourea using the probe GO-ACY, 50 µL of GO-ACY was added to the vials of thiourea (2 mL), and the released dye showed fluorescence, which indirectly offered the concentration of thiourea through the calibration curves as shown in Fig.-1 and Fig.-2. The fluorescence of released acriflavine was measured by the absorption wavelength of 450 nm and emission wavelength of 510 nm.–Upon introduction of analytes such as thiourea or cysteine, the dyes bind to the GO, which will be released due to the competitive binding attraction within the analytes, dye and GO. By this method and from the released quantity of dye, we could evaluate the concentration of analytes, which helped to create a sensitive sensor for thiourea and cysteine in the work. Indeed, the probe has shown high sensitivity towards cysteine in comparison to that of the thiourea, which is more likely due to the presence of the polar thiol nature of the sulfur present in cysteine, whereas the keto–thiol present in the thiourea is less polar than that of the cysteine thiol. Furthermore, under UV flash light (365 nm), cysteine showed the maximum sensitivity limit of 10^{-7} M concentration, and for the thiourea, the sensitivity of visual detection range was found to be above10^{-5} M concentration as shown in the Fig.-2.

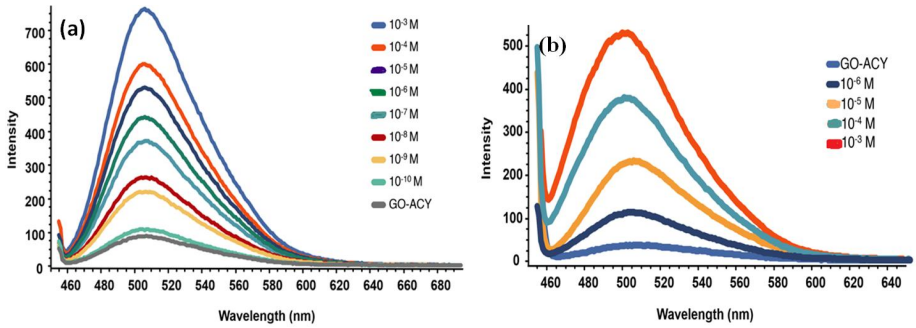

Fig.-1: Sensitivity Studies of Cysteine (a) and Thiourea (b) using the Probe GO-ACY

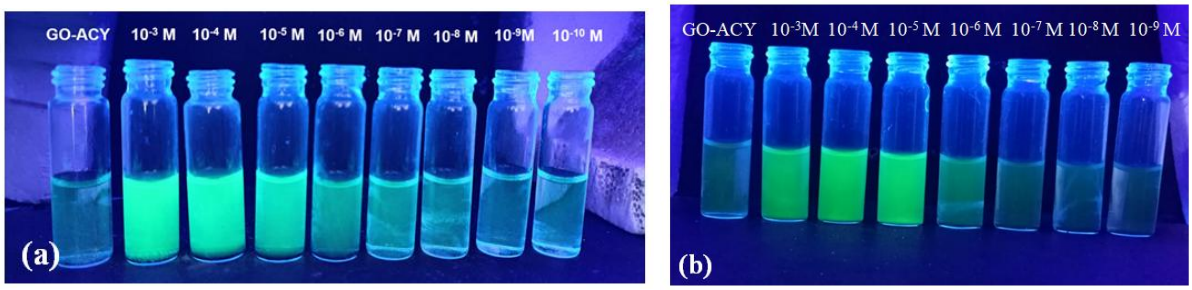

Fig.-2: Naked Eye Detection Method of Cysteine (a) and Thiourea (b) using the Probe GO-ACY under UV Light (365 nm)

Furthermore, the detection limits for cysteine and thiourea were estimated from the corresponding fluorescence calibration plots. Based on the Fig.-3(a), the limit of detection for cysteine using the GO-ACY system was found to be 8.4×10^{-9} M, while for thiourea, a value of 7.1×10^{-7} M was obtained from Fig.-3(b) in aqueous media.

Selectivity of GO-ACY Towards Cysteine and Thiourea

GO-ACY also recognised the thiol derivative, especially the thiourea and the cysteine, while it displayed no response to many of the following analytes chosen. For example, GO-ACY did not respond to aminophenol, ascorbic acid, aniline, glucose, cyclodextrin, potassium fluoride, glutamine, alanine, or cadmium nitrate. When the above-mentioned analytes (2 mL) having the concentration of 10^{-3} M was added to the GO-ACY (50 µL), they did not respond except to the cysteine and thiourea, as shown in Fig.-4.

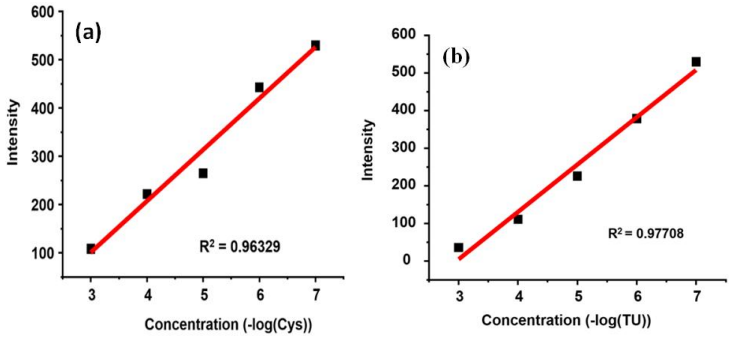

Fig.-3: Linear Regression Analysis at GO-ACY with (a) Cysteine, (b) Thiourea, and LOD was Calculated from the Slope Value of the Graph.

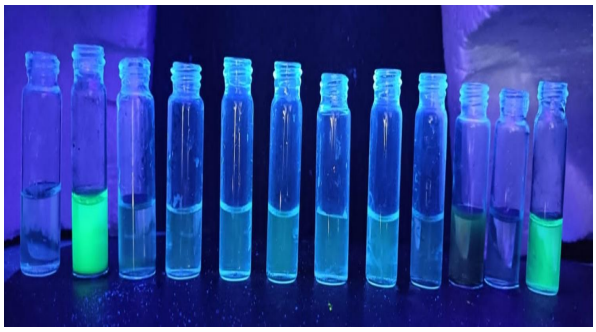

Fig.-4: Selectivity of GO-ACY towards Cysteine and Thiourea. From Left to Right: GO-ACY Interaction with Water. Cysteine, Aminophenol, Ascorbic Acid, Aniline, Glucose, Cyclodextrin, Potassium Fluoride, Glutamine, Alanine, Cadmium Nitrate, and Thiourea

Analyses of Drugs with the Thiourea and Cysteine Unit

GO-ACY effectively recognised the thiourea and cysteine amino acids in water, and it also responded to the commercial pharmaceutical drug molecules having the thiourea or cysteine units integrated in those molecules. As shown in Fig.-5, except for drugs such as carbimazole and N-acetylcysteine all other drugs such as paracetamol, cetirizine, metformin, and thyroxine sodium did not respond to the GO-ACY when examined with 10^{-3} M concentration solutions of all these drugs in water, indicating that the present method can be used in the pharmaceutical industries for detection as well as quantification based on the functional groups of the drugs.[24]

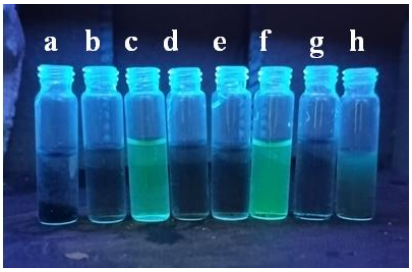

Fig.-5: Response of GO-ACY Towards Pharmaceutical Drugs that Have Cysteine Or Thiourea Moiety (a) Water (blank) (b) Paracetamol (c) Carbimazole (thiourea) (d) Cetirizine (e) Metformin (f) N-acetyl Cysteine (cysteine derivative) (h) Thyroxine Sodium

CONCLUSION

In conclusion, we have developed a facile method of simultaneous detection for the thiourea and cysteine compounds using the supramolecularly loaded acriflavine on the GO materials. Dual detection of cysteine and thiourea has been successfully demonstrated by this work. GO has the potential to adhere to dyes through non-covalent interactions, and the attached dyes will be released upon interaction with cysteine and thiourea. Among them, cysteine displayed a good sensing potential in comparison to that of thiourea

as the LOD reached to the lowest of 8.4×10^{-9} M concentration for cysteine. Furthermore, the detection of cysteine and thiourea can be visualised under the UV flash light, by the interaction of GO-ACY in water. The sensing potential can be extended to the analysis of a few pharmaceutical drugs that contain the cysteine and thiourea moiety, indicating that the GO-ACY has the enormous potential to be utilised in various applications.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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