

# Brilliant Green Decorated Graphene Oxide for the Detection of Cucurbit[7]uril

Ramesh Prakash, Narayanan Selvapalam Govindaraj Usha, Karuppasamy Karpagalakshmi, Lakshminarayanan Piramuthu

**Abstract:** Among the synthetic receptors, Cucurbiturils have gained much attention recent days due to their unique binding potential with variety of drugs and dyes. However, no facile detection method using UV-vis spectroscopy has been developed. Here, we have developed the brilliant green decorated graphene oxide (BGGO) for the detection of cucurbit[7]uril (CB[7]) with good selectivity and sensitivity. Thus, BGGO could able to detect the CB[7] and turn on the release of brilliant green quantitatively. Among the sensors for CB[7], BGGO is the low-cost and sensitive sensor for CB[7] with high selectivity

**Keywords :** CB[7] sensor, graphene oxide, Brilliant green, Cucurbituril.

## I. INTRODUCTION

Cucurbiturils are the class of macrocycles, which have been expanded in many emerging areas of research, especially for the targeted drug delivery materials and for bottom up approach materials that include nanocapsules and dye removal materials. [1]. Macrocycles have been categorized based on the charges of the guests, which can get incorporated into the host molecules. More recently developed Pillarenes, crown ethers and cucurbiturils have the potential to interact with cationic guest molecules, while the other categories of host molecules such as cyclodextrins have the potential to interact with neutral and anionic guest molecules. However, the binding properties of cucurbiturils are very unique and for that purpose they have gained much attention recent days [2]. Based on the structural features of cucurbiturils, they have been explored as portals of the cucurbiturils are built by the rim of carbonyls, while the hydrophobic cavities of cucurbiturils are having the potential to interact with the hydrophobic molecules [3], [4]. The basic building blocks of the cucurbiturils are made up of glycolurils and formaldehyde by the acid-catalyzed condensation, which provided the hydrophobic interior and hydrophilic exterior to attract the molecules of choice (guests) [5], [6]. The size variation of the

cucurbiturils are very different from smaller to large as denoted by the number of glycolurils present in the core of the host molecules [7]. For example, Cucurbit[5]uril can be abbreviated as CB[5] and the available variations are denoted as CB[n], where n = 5 - 8, 10, 14. Although a lots of structural variations have been explored within the CB[n] host molecules, CB[7] has been attracted to scientific community for the reason of abundant solubility in water, through which many biological experiments have been carried out, especially drug delivery related studies and they also showed exceptional binding affinity in water with many biologically important molecules [6, 8]. Due to the large cavity size nature of CB[7] and CB[8], they have explored applications such as drug carrier in biotechnological applications, biomolecule complexation studies, toxicity and taste masking studies and many other biorelated applications. Thus, CB[7] and CB[8] gained much attention in recent studies. Besides, CB[6] and CB[7] also contributed in areas of protein, peptides and amino acids complexation studies [9]. For example, CB[7] made complexation with biologically important molecule such as Insulin and selective complexation of a particular amino acids within the peptide molecules [10]. Among the various host molecules available in the supramolecular field, cucurbiturils could be an alternative to the encapsulation studies of oral and topical drug delivery forms, when compared with the applications that were carried out with cyclodextrins, [11]. Under the wrap of CB[7], molecules such as drugs attained stability and improved the solubility, because drug molecule get complexed with CB[7] to become a single entity [12]. A few important drugs that have been complexed with CB[7] and CB[8] are listed here. For example, an important cancer drug, cisplatin [13] and camptothecin [14], antileprosy drugs for the treatment of lepromatous leprosy such as clofazimine [15], fungicide such as fuberidazole [16], HIV-1 drug such as adefovir, and many other drugs such as bis(L-phenylalanine propyl)ester [17], ranitidine and fasudil, [18] have been examined. All these studies indicate the role of CB[n] in the medicinal and material science areas and thus detection of CB[n] should be important, because they could not be recognized or visualized by the UV and other staining agents, except Iodine, which also displayed very weak signals/spot under various environments.

Unlike the other organic molecules, identification and quantification CB[n] or CB[7] is very challenging. Without the aid of maldi-tof and NMR, it would be very difficult to trace the quantity of CB[n] by any other affordable techniques,

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such as TLC and UV-vis spectroscopy, which are commonly employed by the organic chemist to trace the organic molecule qualitatively. Therefore, developing a facile visible dye based sensor would support the detection of CBs for in-vivo and in-vitro applications to detect the presence of CBs. Furthermore, visible dye based CB sensor would also support the synthesis of functionalized CBs, for which we require a facile sensor for the laboratory use. Recently, our research group have published a CB sensor based on a rhodamine B decorated copper nanoparticle by the method of fluorescence turn-on technique [19]. In a similar way, we have explored the utilization of graphene oxide as a core material, on which rhodamine B was bound non-covalently. This graphene oxide decorated rhodamine material could detect very selectively CB[7], among the various members of CB host family members [20]. However, it required expensive fluorescence spectrophotometer for the quantification of CB[7] and that led to explore other inexpensive ways to quantify the CBs, especially for the CB[7]. So, we decided to change the fluorescent dye that we used to decorate the graphene oxide by the non-fluorescent dye to explore the same hypothesis, which would require only visible spectrophotometer and such instruments are the most inexpensive one. Among the various dyes existing, we have selected brilliant green (BG), because it is an inexpensive dye with high intensive color, even at the lowest concentration [21]. Brilliant green is expected to get absorbed on graphene oxide by the non-covalent interactions through the  $\pi$ - $\pi$  interactions of the aromatic groups of dye with the graphene oxide [22, 23]. As expected, brilliant green decorated graphene oxide (BGGO) also successfully released the BG upon interaction with the CBs, indicating that BGGO could be the choice of material for sensing the CBs in a more affordable way. Therefore, the sensor may provide a facile and affordable way for the determination of CB[7] by the usage of BGGO. Similar to cyclodextrins, CB[7] has been explored to make complexation with many of the pharmaceutically important drugs, which are expected to be available in the market in the next century and an affordable sensor would be useful for the pharma industry for the determination of CB[7]. Thus, our method of determination of CB[7] using BGGO would be an useful technique for the laboratories of pharma industry. Besides, during the synthesis and isolation of functionalized CB[7], it is hard to measure the presence of CB[7] in the water solution, because CB[7] does not possess any chromophoric groups on it. For this reason, BGGO would provide an opportunity to measure CB[7] in the solution very conveniently. Overall, the present sensing method of CB[7] would help the supramolecular chemists and the pharma industry in many ways in the future.

## II. RESULT AND DISCUSSION

BGGO is the key material that has been prepared based on our previous work that we published by replacing the dye of rhodamine B by brilliant green [20]. The core material graphene oxide was prepared by the modified hummers method, which was also mentioned in detail in our previous work [20]. Here, we have mentioned briefly the procedure to understand the preparation method of BGGO. The core

material graphene oxide was prepared from the commercial source of graphene by using the oxidants such as  $\text{KMnO}_4$ ,  $\text{NaNO}_3$  and hydrogen peroxide in water. The water soluble portion of graphene oxide was extracted and concentrated to obtain the water soluble graphene oxide. For example, 2 g of graphene was treated with  $\text{NaNO}_3$  in ice bath and subsequently digested with 90 mL sulfuric acid and stirred it for 4 h, which peeled the graphite to single or multi layers graphene oxidative product, which is water soluble product. Afterwards, 12 g of  $\text{KMnO}_4$  was added to oxidize the peeled graphene oxide at temperature below  $15^\circ\text{C}$  and it was further diluted with 184 mL of water and stirred further 2 h in ice bath and 2 h at  $35^\circ\text{C}$ . It was later boiled at  $98^\circ\text{C}$  for 15 min and the solution become dark brown. To this, 40 mL of hydrogen peroxide and 400 mL water were added, which changed the solution to yellow. The product obtained was treated with 10 % HCl and neutralized by multiple washing with DI water. The final product's volume was adjusted to 100 mL and retained this solution for further studies [20].

Brilliant green adhered to the graphene oxide and obtained the BGGO. In brief, 96 mg of brilliant green (1 mM) and 4 mL of graphene oxide were dissolved in 200 mL water. To that, 700  $\mu\text{L}$  of hydrazine hydrate (30%) was added and stirred at room temperature for 12 h for the brilliant green to get adsorbed on graphene oxide. It was stored in dark for 72 h and the unbound brilliant green was removed by multiple water

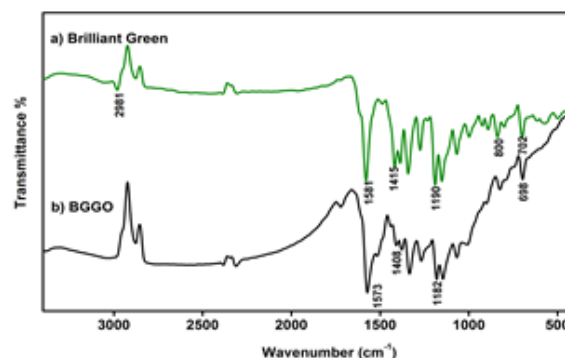


Fig 1. FTIR Spectrum of brilliant green decorated graphene oxide - BGGO

wash. Furthermore, it was treated with 1 M NaOH to achieve the turn off the dye release from the graphene oxide. Final volume of the stock solution prepared by the addition of 10 mL of DI water was centrifuged product of BGGO. BGGO has been characterized by SEM and FT-IR. The presence of brilliant green on graphene oxide can be assured by the FTIR spectroscopy as shown in fig.1. Some of significant peaks of brilliant green have been displayed in the FTIR spectrum of BGGO with a slight shift and the peaks such as 1581, 1415, 1190  $\text{cm}^{-1}$  represents C=C stretching vibration of benzene and the aromatic C-N stretching vibrations. These peak values indicate the assembly of brilliant green on the graphene oxide. peculiar compare to the other graphene oxide related SEM images. In the case of rhodamine B attached graphene oxide, it showed like a flower like structure, whereas the BGGO appeared like a branch of the tree.

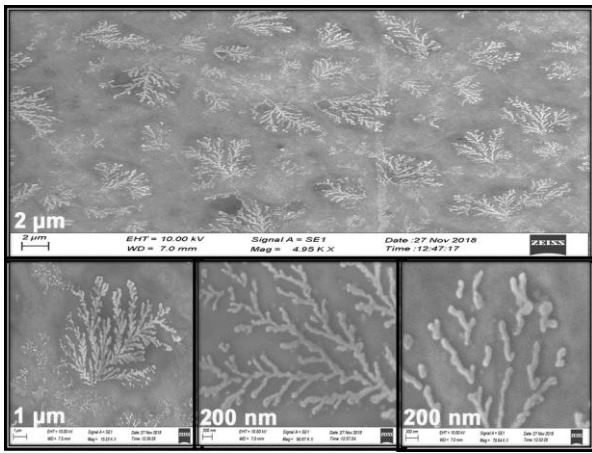


Fig 2. SEM images of BGGO under different magnification showing the dendrites like structures

Unlike the rhodamine B attached graphene oxide, the SEM image of the BGGO was quite different as shown fig. 2. The SEM of the BGGO displayed as dendrites like structure, which shown like a branches of a tree and that is very much unusual.

Next, we explored the turn on release of brilliant green sensing potential of the BGGO in favor of CB[7]. For that, we have prepared the solutions of CB[7] with various concentrations. We have prepared solutions of CB[7] in the concentration range of  $10^{-2}$  to  $10^{-7}$  M in phosphate buffer. We prepared seven different vials with 3 mL volume of CB[7] in the range of  $10^{-2}$  to  $10^{-7}$  M and to that 200  $\mu$ L of BGGO was added and measured the intensity of the absorbance of the released brilliant green dye. We have plotted the graph of the collected data.

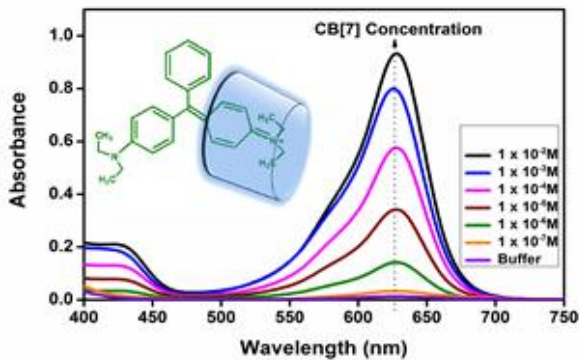


Fig 3. UV-Vis spectra of BGGO upon addition of CB[7] concentration ( $10^{-2}$  to  $10^{-7}$  M).

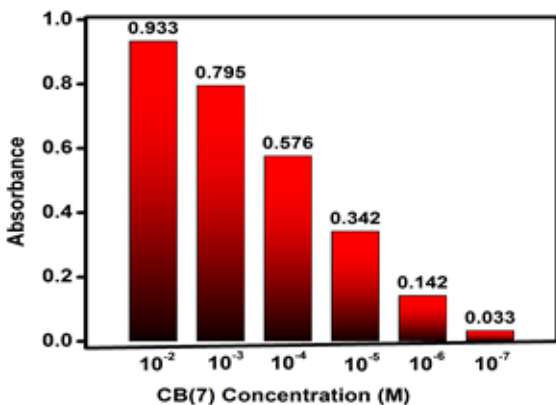


Fig 4. Quantitative increase of absorbance upon addition of CB[7] at various concentration.

We prepared seven different vials with 3 mL volume of CB[7] in the range of  $10^{-2}$  to  $10^{-7}$  M and to that 200  $\mu$ L of BGGO was added and measured the intensity of the absorbance of the released brilliant green dye. We have plotted the graph of the collected data. As shown in fig.3, when the concentration was increased for CB[7], the absorbance also increased, indicating that the turn on release of brilliant green is proportional to the concentrations of CB[7]. From the fig. 3 and fig. 4, we could understand the relationship between the concentration of CB[7] with respect to the quantum of release of brilliant green and that allowed to prepare the calibration curve for the CB[7]. As shown in fig. 5, the calibration curve indicated that the unknown concentration could be conveniently measured. Besides, the regression value of the slope was also supportive for the estimation of CB[7] of the unknown sample

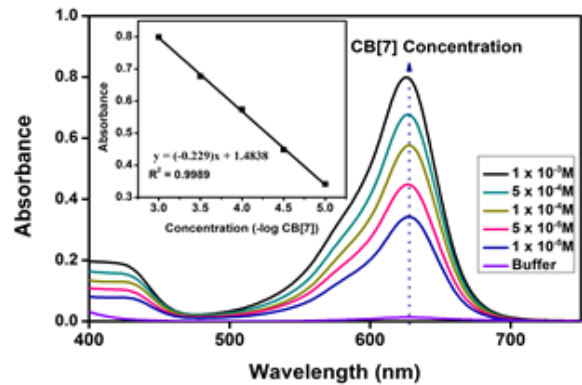


Fig 5. UV-Vis spectra of BGGO with CB[7] at various concentration. Inset: Concentration of CB(7) plotted against the absorbance of BGGO for CB[7] ( $\lambda_{max}$  = 628 nm).

Based on that, we have also developed the naked eye detection of the CB[7] for the different concentrations As shown in fig. 6, various concentrations of CB[7] could be seen, by the addition of BGGO. From that we could see the concentration of CB[7] in the range of  $10^{-2}$  to  $10^{-7}$  M. However, concentration of CB[7] in range between  $10^{-2}$  M to  $10^{-5}$  M, we could visually measure the concentration without much difficulty, indicating that this method could be useful to measure concentration of CB[7] above  $10^{-4}$  M by visual method.

To understand the selectivity of this method, we prepared the solutions of CB[5], CB[6] and CB[7] in phosphate buffer in the concentration of  $10^{-4}$  M. In three different vials, 3 mL volume of CB[5], CB[6] and CB[7] were taken and to that 200  $\mu$ L of BGGO was added in each vial. As shown in fig. 7. CB[5] and CB[6] did not release the brilliant green; but CB[7] containing vial shown the release of brilliant green considerably, indicating the selectivity of the method, which is specific to the host molecule – CB[7]. Thus, this method is beneficial for the sensing of CB[7] and helpful to detect the concentration of CB[7] to the lowest of  $10^{-4}$  M under the visible light. This method does not require any expensive equipment to visualize the CB[7], which could be useful during the isolation of CB[7] and its derivatives

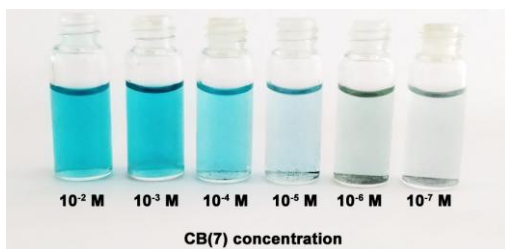


Fig 6. Naked eye detection of CB[7] (10<sup>-2</sup> to 10<sup>-7</sup> M) using BGGO under visible light.

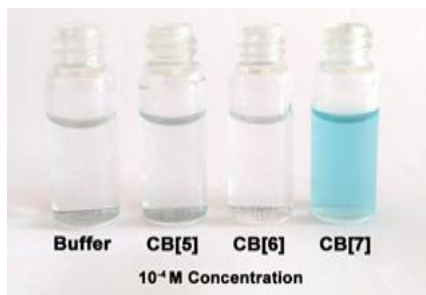


Fig 7. Selectivity of BGGO towards CB[7] by a naked eye detection method.

### III. CONCLUSION

From the inexpensive brilliant green dye, BGGO has been synthesized, characterized and examined successfully for the detection of cucurbit[7]uril with high selectivity. The binding affinity and the water soluble property of CB[7] made this turn-on release of brilliant green process possible. Furthermore, BGGO has displayed high sensitivity for CB[7] with the maximum sensitivity of 10<sup>-6</sup> M by the UV-Visible spectroscopy. Besides, naked eye detection method provided an opportunity to detect the CB[7] with much less effort. The selectivity of CB[7] observed by naked eye detection will contribute the sensor for easy identification of CB[7] during isolation of CB[7] or its derivatives. Overall, the present method will provide a facile detection method for CB[7] in a more economical way.

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