Electronic Supplementary Material (ESI) for Analytical Methods.


# Colorimetric Detection of Hydrogen Peroxide and Glucose using Brominated Graphene

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## Experimental

### Materials

Hydrobromic acid (HBr; Sd fine chemicals, India), phosphoric acid ($H_3PO_4$; Qualigens, India), 95% sulphuric acid ($H_2SO_4$; Fischer Scientific, India), potassium permanganate ($KMnO_4$; Qualigens, India), 30% hydrogen peroxide ($H_2O_2$) (Merck, India), hydrochloric acid (HCl; Fischer Scientific, India), methanol ($CH_3OH$; CDH, India), acetone ($CH_3COCH_3$; CDH, India), D-Glucose ($C_6H_{12}O_6$, Fischer Scientific, India), Glucose oxidase (Sigma Aldrich, USA), sodium hydrogen phosphate ($Na_2HPO_4$; Merck, India) and potassium hydrogen phosphate ($K_2HPO_4$; Merck, India) were used as received, Dry ethanol was prepared by leaving ethanol (Saraya Distilliary, India) over CaO for overnight followed by distillation over fresh CaO. Graphite was provided as a gift by Dr. Sanjay Dakate of National Physical Laboratory, New Delhi. Serum samples were collected from faculty of Ayureveda, Institute of medical science, Banaras Hindu University.

**Synthesis of Graphene Oxide (GO)**

Typically, 400 mL 9:1 (v/v) mixture of concentrated $H_2SO_4$/$H_3PO_4$ was added to 3.0 g graphite powder taken in a round bottom flask containing teflon-coated magnetic stirrer and stirred for 2 h at room temperature. $KMnO_4$ (18.0 g, 6 wt equiv to graphite) was added to the resultant mixture leading to a slight increase in temperature to 35 - 40 °C. The reaction mixture was then heated to 50 °C, and stirred for 12 h. The final reaction mixture was cooled to room temperature and poured onto 400 mL ice-cold water containing 3 mL 30% $H_2O_2$ . The resultant paste like product was then washed by redispersing and centrifugation successively with 200 mL 5% HCl, 200 mL ethanol, and then with deionised water for several times till the supernatant becomes neutral. Finally, it was redispersed, and centrifuged twice with acetone (to remove water from GO) and dried under vacuum at 40 $^oC$. The synthesized graphene oxide (GO) was obtained as a dark colored solid.

**Synthesis of Brominated Graphene (GBR)**

GO (150 mg) was added to HBr (7 mL, 48 % extra pure) taken in a round bottom flask during sonication, and then transferred to an oil bath maintained at 122 $^oC$ and kept under reflux for 5 h. The resultant product was then precipitated in water, filtered and washed with double distilled water (250 mL) and finally with methanol (250 mL). Resultant product was dried under vacuum at 50 $^oC$ for 24 h.

**Table S1. C**omparative results of $K_m$ between GBR and HRP.[22]

| Catalyst | Substrate | $K_m$ (mM) |
|---|---|---|
| GBR | TMB | 0.83 |
| GBR | $H_2O_2$ | 10.98 |
| HRP[22] | TMB | 0.15 |
| HRP[22] | $H_2O_2$ | 0.27 |

Ref 22. W. Shi, Q. Wang, Y. Long, Z. Cheng, S. Chen, H. Zheng, Y. Huang, *Chem. Commun*. 2011, **47**, 6695–6697.

**Fig. S1.** Photograph of the vials containing GBr (100 $\mu$L, 1 mg/mL), TMB (50 $\mu$L, 1 mM) and varying concentration of $H_2O_2$ (200 $\mu$L 0.5 - 10 mM) taken in PBS (*p*H = 4.48, 200 $\mu$L) buffer solutions.

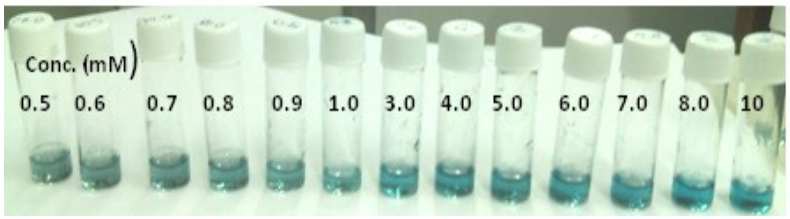



**Fig. S2.** Selectivity study towards analytes (urea, thiourea, citric acid, uric acid and glucose) (each 50 µL, 50mM) [Condition: analytes (50 µL, 50 mM) and Glucose oxidase (50 $\mu$L, 2.5 mg/mL) was added into PBS (100 $\mu$L, *p*H 7.0) incubated at 37°C for 30 min, later transfer to the solution containing GBR (100 $\mu$L, 1 mg/mL) and TMB (50 $\mu$L, 1 mM) in PBS buffer solutions (200 $\mu$L, *p*H 4.48) and kept for 1 h in dark at 30 °C. The absorbance spectra were monitored at 652 nm (corresponds to the oxidised TMB)]

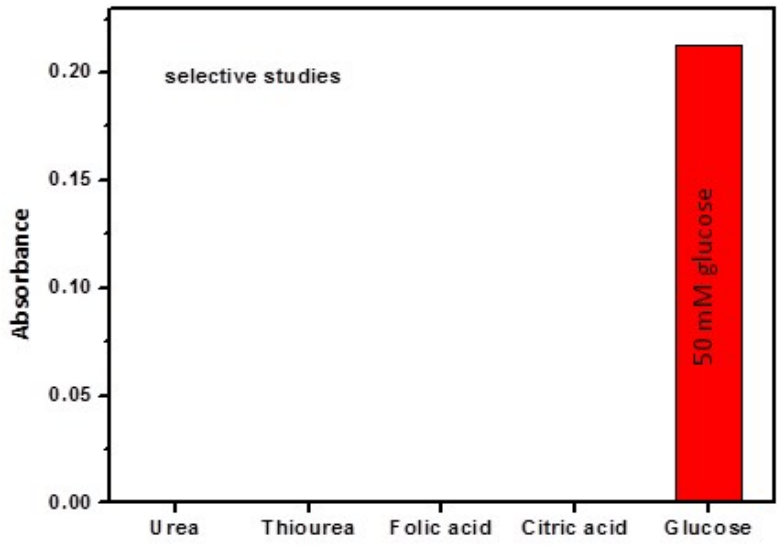



**Fig. S3.** Interference study of analytes (urea, thiourea, citric acid and uric acid) (50mM each in sensor solution). [Condition: glucose (50 µL, 50 & 100 mM) and glucose oxidase (50 $\mu$L, 2.5 mg/mL) has been added into PBS (100 $\mu$L, *p*H 7.0) incubated at 37°C for 30 min, later transferred to the sensor solution containing GBR (100 $\mu$L, 1 mg/mL), TMB (50 $\mu$L, 1 mM) and interfering analytes (50 mM each in sensor solution) in PBS buffer (200 $\mu$L, *p*H 4.48) and kept for 1 h in dark at 30 °C. The absorbance spectra were monitored at 652 nm (corresponding to the oxidised TMB).] [Inset: results of standard samples with similar concentration of glucose.]

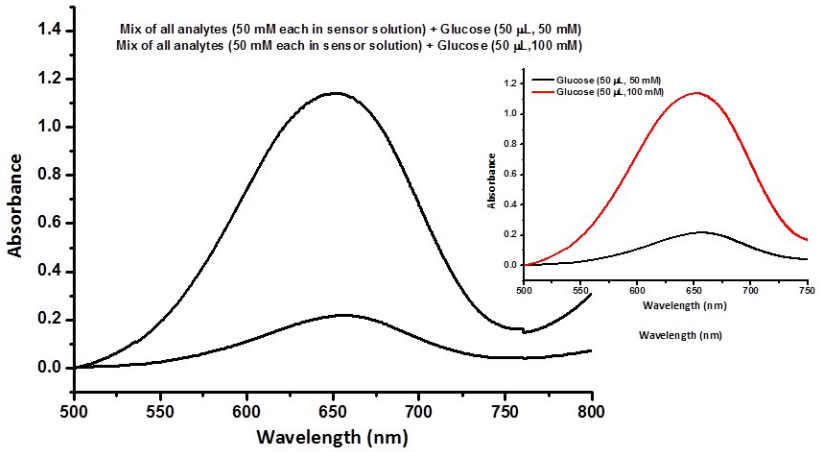
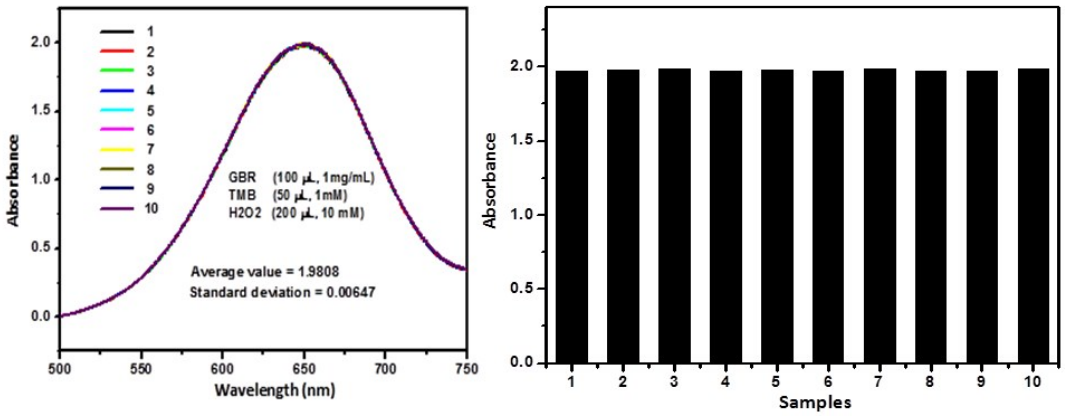


**Fig. S4.** Reproducibility experiments performed 10 times using same set of reaction ` parameters: GBR (100 $\mu$L, 1 mg/mL), TMB (50 $\mu$L, 1 mM), $H_2O_2$ (200 $\mu$L, 10 mM) conducted in PBS (*p*H 4.48, 200 $\mu$L)




**a** **b**

**Table S2.** Validation of the developed strategy (glucose detection) by standard addition experiment

| Sr. No. | Glucose spiked (mM) | Glucose Measured (mM) | Recovery % | Standard Deviation (SD)[a] |
|---|---|---|---|---|
| 1 | 40 | 39.92 | 99.80 | 0.60 |
| 2 | 50 | 50.10 | 100.2 | 0.30 |
| 3 | 70 | 69.90 | 99.85 | 0.50 |

[a] each same sample was repeated 3 times (N= 3)

**Table S3.** Comparative results of glucose determination in serum samples using auto analyzer [Erba, Mannheim, XL system packs, Germany, used in Institute of Medical Science, BHU] (based on enzymatic reaction) and the proposed colorimetric method

| **Measured by** | **Sample 1** | **Sample 2** | **Sample 3** |
|---|---|---|---|
| Auto analyser (mM) | 6.90 | 12.30 | 16.60 |
| **Proposed method** | 6.57 ± 0.60[a] | 11.72 ± 0.70[a] | 16.08 ± 0.40[a] |

[a] Standard deviation (as each same sample was repeated 3 times) (N= 3)