Graphene Oxide and Pluronic Copolymer Aggregates –Possible Route to Modulate the Adsorption of Fluorophores and Imaging of Live Cells

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(1) Cell Preparation: HaCaT (Human Keratinocyte) cell was prepared following standard methodology.[76] For the cell incubation, 200 μl of HaCaT cells (ca. $5x10^5$ cells ml^{-1}) were incubated with 0.20 μg ml^{-1} of GO and R6G with or without 5wt% P123 micelle loading in cellular medium for 30 minutes. Cells were washed three times with PBS buffer to remove any unbound R6G-GO or P123-R6G-GO accordingly before fluorescence imaging.

(2) Inner filter effect correction:

GO strongly absorb light in the range of 270-360 nm and in our experiments, all the probe molecules are excited at 408 nm which slightly overlap with the absorption spectra of GO. So the decrease in the emission intensity of the probe molecules after addition of GO could be due to the "inner filter effect". This effect refers to the absorbance of light at the excitation or emission wavelength by the compounds present in the solution. Therefore, it is necessary to consider this effect before discussion the interaction between probe molecules and GO. Fluorescence intensity was collected from the centre of the cuvette and thus the inner filter effect can be estimated from the following equation.

$$F_{obs} = F_{corr} \, X \, 10^{-\frac{A_{ex} * d_{ex}}{2} - \frac{A_{em} * d_{em}}{2}} \quad (1)$$

where F_{obs} is the measured fluorescence intensity and F_{corr} is the corrected fluorescence intensity in absence of inner filter effect, d_{ex} and d_{em} are the cuvette path length in the excitation and emission direction (in cm), respectively, A_{ex} and A_{em} are the measured absorbance value at the excitation and emission wavelength with addition of GO, respectively.

After the correction, $\frac{F_0}{F}$ is plotted against the concentration of GO and we found that there exist a significant interaction between the probe molecules and GO. Thus, we have plotted each Stern-Volmer plot after removing the inner filter effect.

(3) Determination of free dyes (C_e): The intensity of the emission spectra of the fluorophores are proportionally decreased upon addition of GO. The association of fluorophores with GO can be represented by the following equation.

$$\text{GO + Fluorophore} \leftrightarrow \text{GO-Fluorophore} \tag{2}$$

$$K_b = \frac{[GO-Fluorophore]}{[GO]\,[Fluorophore]} \tag{3}$$

Where K_b is the association constant. Now, if $C_{Fluorophore}$ is the total concentration of the fluorophores then,

$$C_{Fluorophore} = [\text{Fluorophore}] + [\text{GO-Fluorophore}] \tag{4}$$

Combining equation (12) and (13) we obtain,

$$\frac{C_{Fluorophore}}{[\text{Fluorophore}]} = 1 + K_b\,[GO] \tag{5}$$

If we assume that the fluorescence intensity is proportional to the concentration of the free fluorophores then,

$$\frac{F_0}{F} = 1 + K_b\,[GO] \tag{6}$$

As in our study, we have observed that the quenching process is static one and thus in the stern-volmer equation, one can replace K_{SV}, Stern-Volmer constant with K_b, the binding constant. Thus, following the equation (4) and (5), we obtain the free dye concentration in the solution.

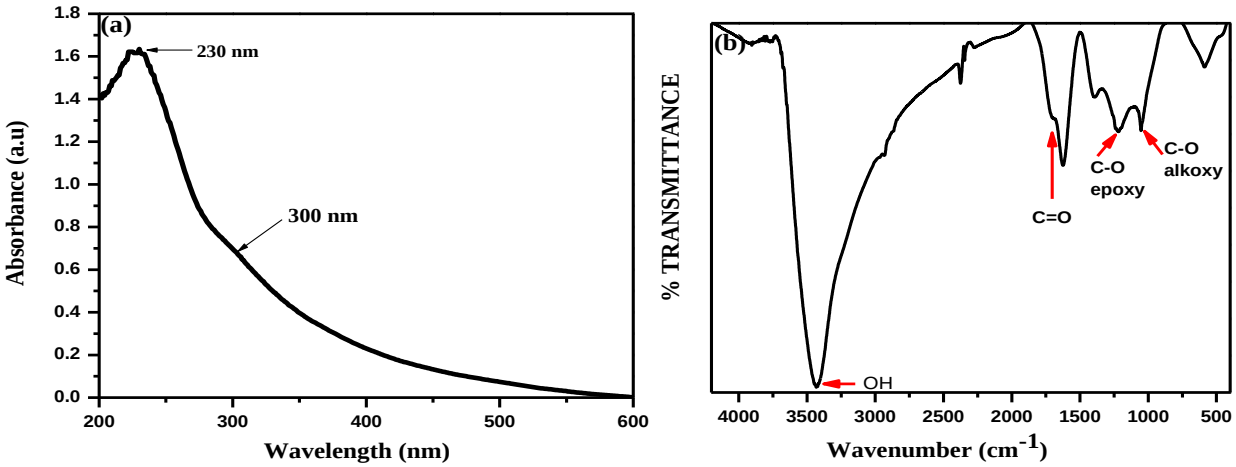

Figure S1. (a) UV-Visible Absorption Spectra and (b) FTIR Spectra of synthesized GO.

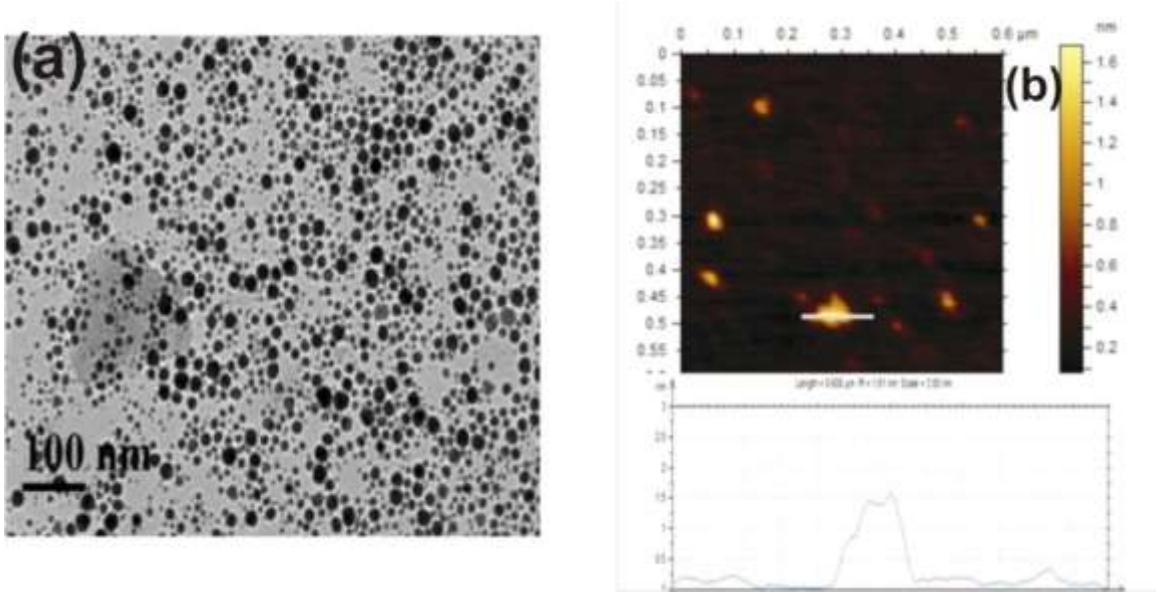

Figure S2. (a) TEM image of 5wt. % P123 micelle (Scale bar shows 100 nm) and (b) Tapping modes AFM image of GO deposited on a freshly cleaved mice surface and height profile diagram of GO flakes.

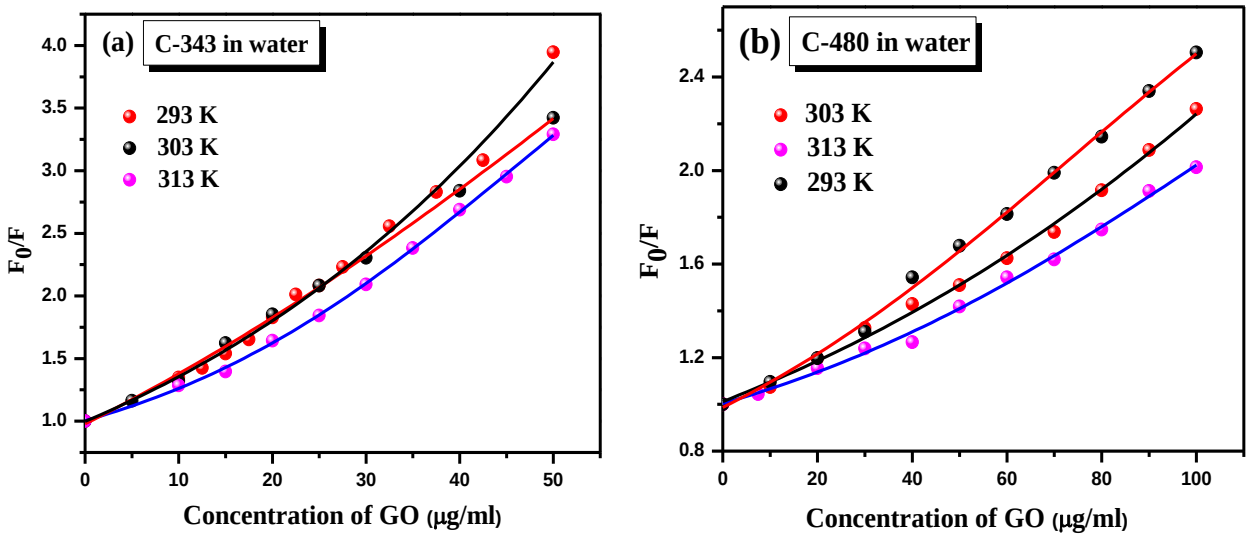

Figure S3. Stern-Volmer plot of C-343 and C-480 in water in presence of different concentration of GO in three different temperature.

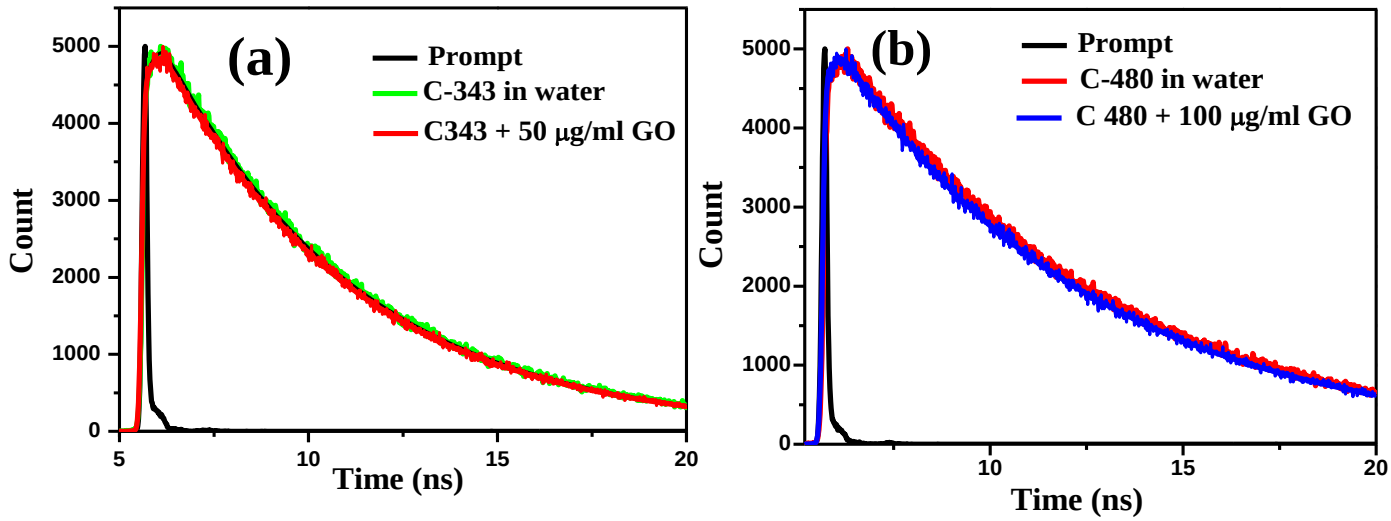

Figure S4. Fluorescence Lifetime of (a) C-343 and (b) C-480 in water in presence of different concentration of GO

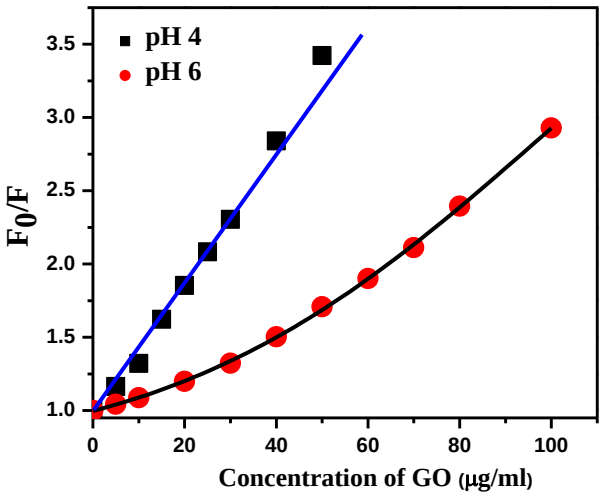

Figure S5. Stern−Volmer plot of C-343 against the concentration of GO at pH 4 (black line) and 6 (red line).

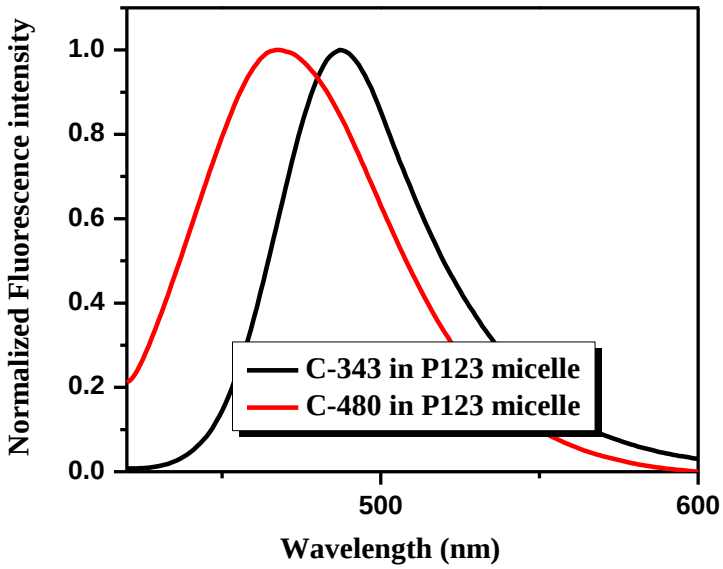

Figure S6. Normalized Emission Spectra of C-343 and C-480 in 5wt% P123 micelle.

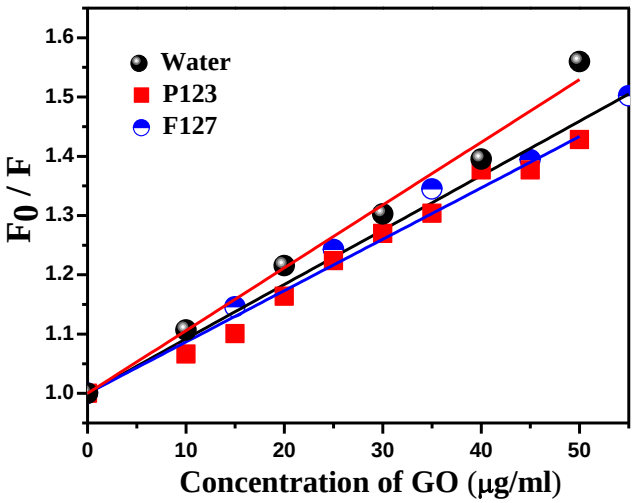

Figure S7. Stern-Volmer plot of C-343 in different environments in presence of different concentration of GO.

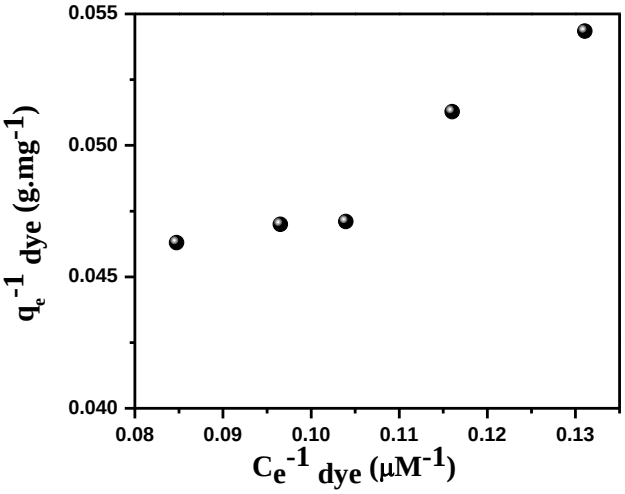

Figure S8. Adsorption isotherm of C-480 in P123 micelle adsorbed on GO surface.

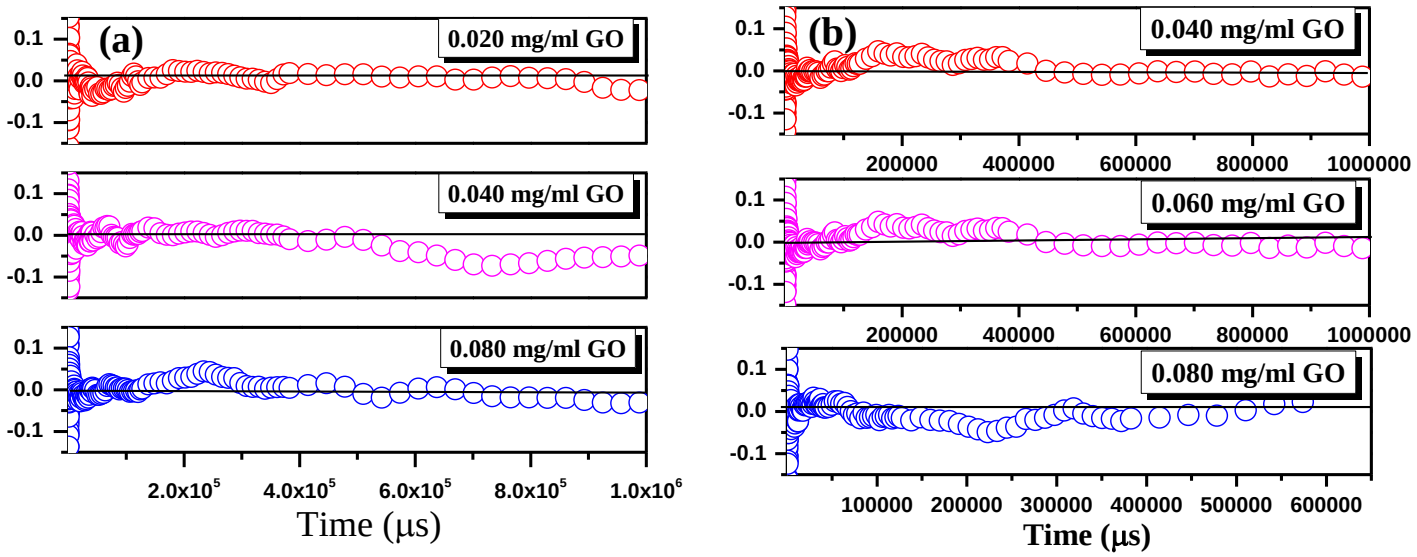

Figure S9. Residuals of the fitted FCS traces of (a) C-343 and (b) C-480 in water in presence of different concentration of GO.

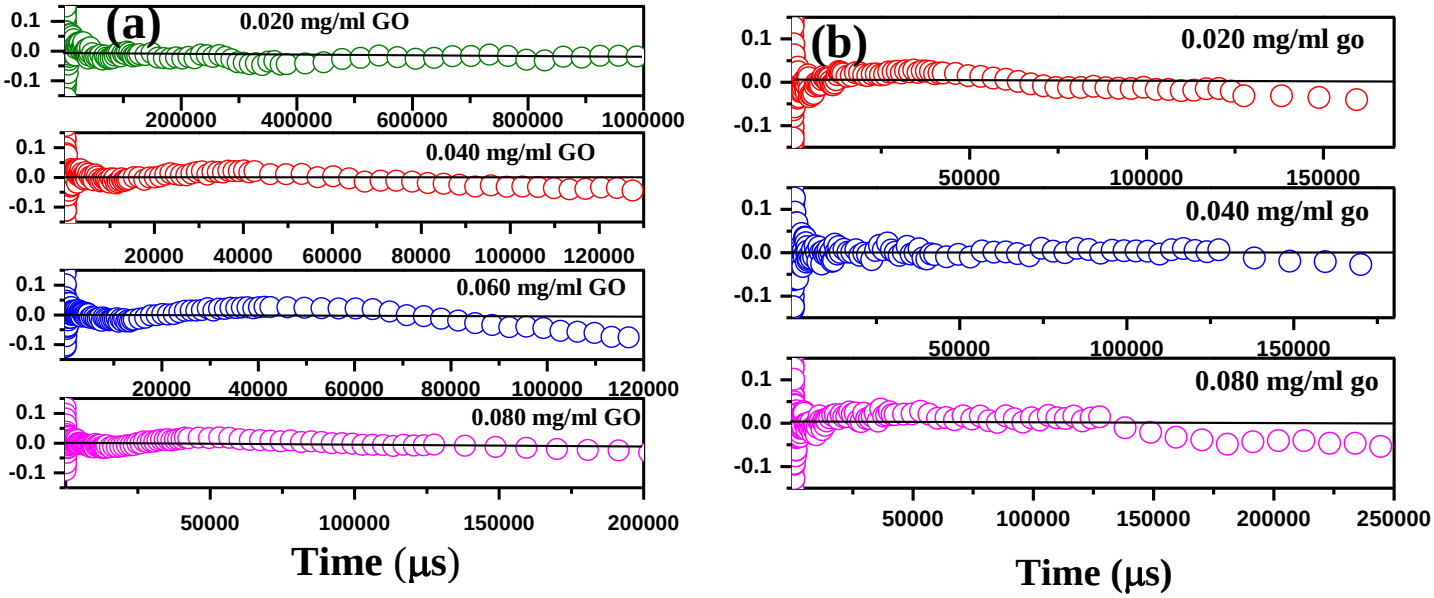

Figure S10. Residuals of the fitted FCS traces of (a) C-343 and (b) C-480 in P123 micelle in presence of different concentration of GO.

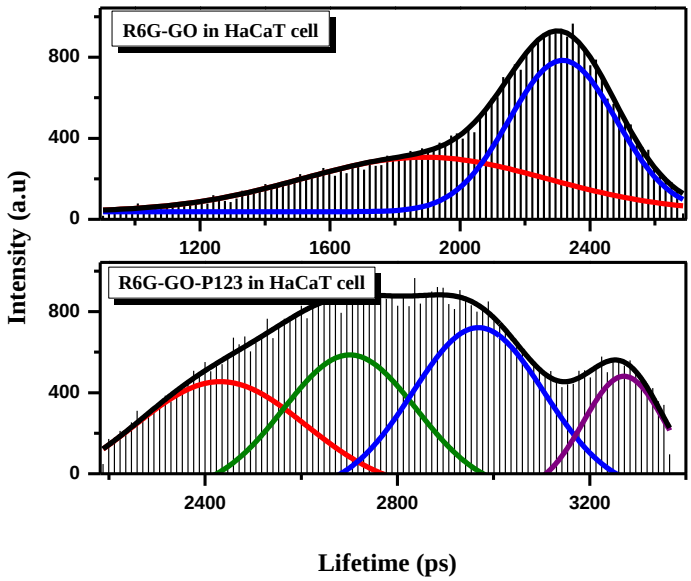

Figure S11. Fluorescence Lifetime distribution plot of the HaCaT cell in fresh cell culture media after incubated with R6G-GO (top) and R6G-GO-P123 (bottom).