Supporting Information

Room temperature and solvothermal green synthesis of self passivated carbon quantum dots

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Electronic Supplementary Material (ESI) for RSC Advances

1. Experimental Section:

1.1. Materials and Methods.

Polyethylene glycol (M.W. 200; PEG-200), acetone, ethanol, sodium hydroxide, ammonia, dimethyl amine, triethyl amine, hydrochloric acid and potassium hydroxide were purchased from Merck (India). All the chemicals were of analytical grade and used without further purification. For biological experiment milli-Q grade water was used throughout.

1.2. Room temperature synthesis of CQDs.

In a typical synthesis of carbon quantum dots (CQDs), 5 mL of PEG-200 was mixed with 1 mL of 0.1 M NaOH solution. The mixture was shaken well to produce a homogeneous solution which was allowed to sonicate in a sonication bath for 1 hour. During this time a distinct yellow colored solution was formed, which was kept undisturbed for overnight to yield CQDs with a characteristic yellowish brown color.

1.3. Variations in reactants.

In this study NaOH was replaced by other basic substances keeping the other procedures unaltered. Briefly 1 mL of ammonia, dimethyl amine, triethyl amine, potassium hydroxide was mixed separately with 5 mL of PEG-200. The mixtures were sonicated for 1 hour in a sonication bath followed by keeping them overnight undisturbed. The resultant solutions were monitored by UV-VIS absorbance spectrophotometrically.

1.4. Concentration of base.

In order to justify the proper amount of base required for a particular reaction, concentration of base (sodium hydroxide) was varied from 0.1 M, 0.5 M, 1 M, 2 M and 4 M. Briefly, 1 mL of base of above mentioned concentration was mixed with 5 mL of PEG-200 and sonicated for 1 hour followed by keeping them undisturbed at room temperature for overnight. The solutions were monitored by UV-VIS absorbance spectrophotometrically.

1.5. Low temperature green synthesis of CQDs.

In this process a low temperature 'green' solvothermal route was used to synthesize CQDs. In a typical process about 20 mL of PEG- 200 was mixed with 5 mL of concentrated sodium hydroxide solution to produce a homogeneous solution. This homogeneous solution was then subjected to solvothermal heating at 160°C for 24 hours to produce CQDs.

1.6. Toxicological study on human red blood corpuscles (RBC).

Human erythrocyte enriched fraction was centrifuged twice (3,000 rpm, 15 minutes) at 4°C to removed the residual plasma and buffy coat. RBCs were washed 3 times with sodium phosphate buffer (pH 7.4) and resuspended in same buffer to make packed cell volume of ~10% (w/v) as stock. Then haemolysis and cell labeling was carried out by mixing 690 µL of the above RBC stock suspension with different volume (0 - 400µL) of CQDs dispersion and making a total volume 1.5 mL by addition of phosphate buffer saline to maintain 5.5% cell volume for each and every set of experiment. Afterward, the erythrocyte cells were centrifuged (3,000 rpm, 15 minutes) at 4°C and washed thrice with sodium phosphate buffer. Haemolysis was monitored UV-VIS spectrophotometrically at 540 nm, same solution was used for bioimaging.

1.7. MTT assay on Hep G2 cell lines.

We performed MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays on human liver carcinoma Hep G2 cell lines and the cell viability was recorded after exposure to CQDs at different concentrations up to 24 h. Cells were seeded into 96-well microtiter plates at a density of 5×10^5 per well and allowed to adhere for 24 h. Then CQDs were introduced at final concentrations between 0-16µL into 8 wells for each concentration. After incubation, cells were washed with PBS twice and incubated with MTT solution (450µg/mL) for 3-4h at 37 ^{0}C. The resulting formazan crystals were dissolved in an MTT solubilization buffer and the absorbances were measured at 570 nm by using a microplate reader (Biorad) and the values were compared to the control cells.

2. Characterizations:

Fourier Transform Infrared Spectroscopy (FT-IR) was conducted by Perkin-Elmer spectrum RX-1 IR spectrophotometer by using methanolic dispersion of CQDs. For absorption and fluorescence measurements Shimadzu absorption spectrophotometer (model no: UV-1700) and Hitachi spectrofluorimeter (model no: F 7000) was used respectively; photostability and quantum yield was obtained by using the same spectrofluorimeter. High-resolution transmission electron microscopy (HRTEM) was carried out by using JEOL 2100, operating at an acceleration voltage of 200 kV. Surface topology of CQDs was obtained by using AFM (Vecco Metrology Inc.) in tapping mode. For AFM analysis aqueous dispersion of the test sample was drop casted on a cleaned glass slide and dried in air. For HR-TEM analysis a very dilute alcoholic suspension was prepared, which was then deposited on a carbon coated copper grid and finally dried in air. Phase characterization of the sample was carried out by using PANAlytical xpert pro X-ray diffractometer with Cu-Kα -targets at 2mm slits. Zeta potential of the CQDs were measured by using Malvern zetasizer while Time Correlated Single Photon Counting (TCSPC) lifetime measurement was carried out by using a picosecond diode laser at 370 nm (IBH, Nanoled) as a light source and the signal was taken at magic angle (54.7^{o}) polarization using an Hamamatsu MCP PMT (3809U). The data analysis was evaluated by using IBH DAS, version 6, and decay analysis software. Fluorescence microscopic image was obtained by using Carl Zeiss-Axiolab fluorescence microscope while confocal microscopic image was obtained by using Nikon A1 confocal microscope.

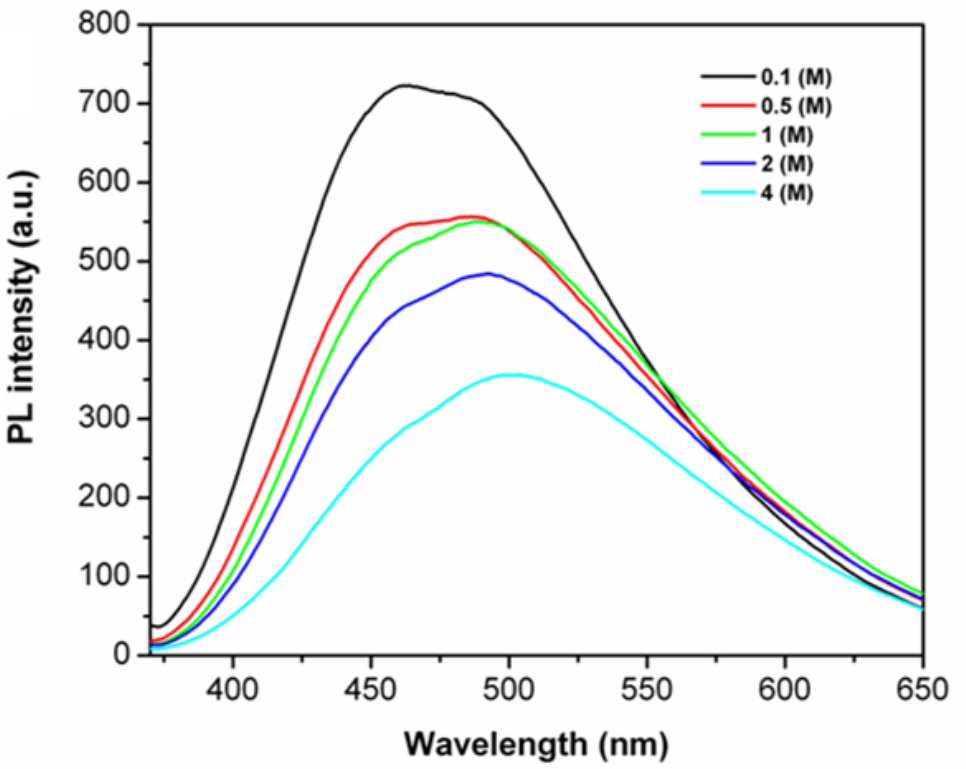

Figure S1. PL intensity of room temperature synthesized carbon quantum dots against different concentration of base.

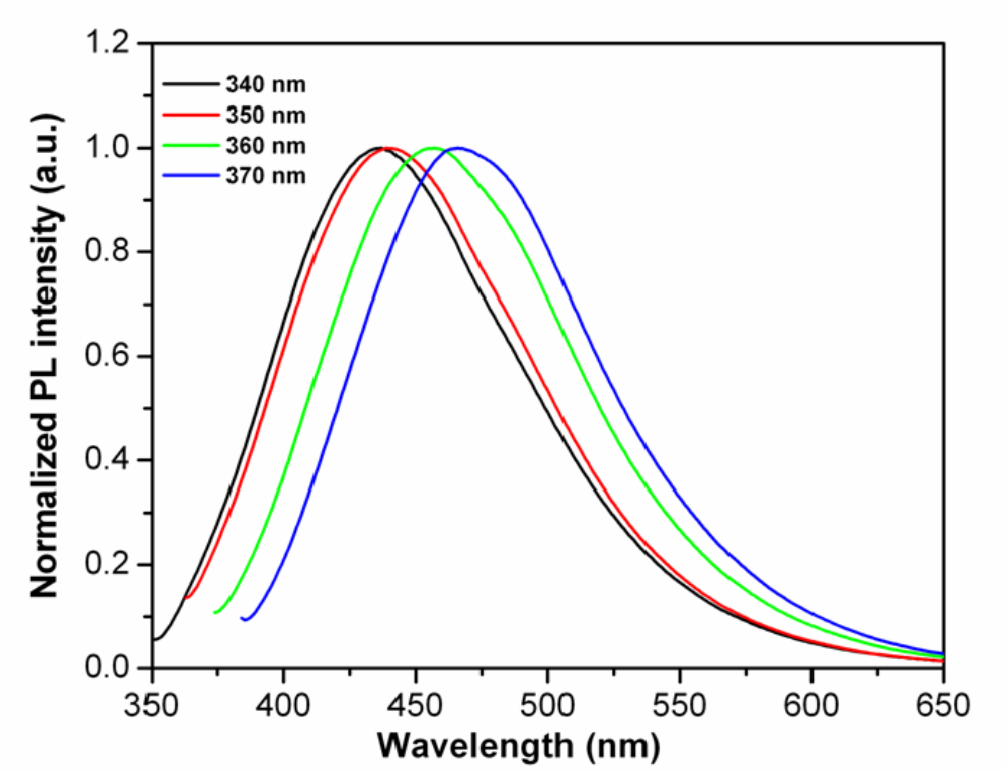

Figure S2. Normalized PL intensity of solvothermally synthesized CQDs at different wavelength; a gradual red shift was observed.

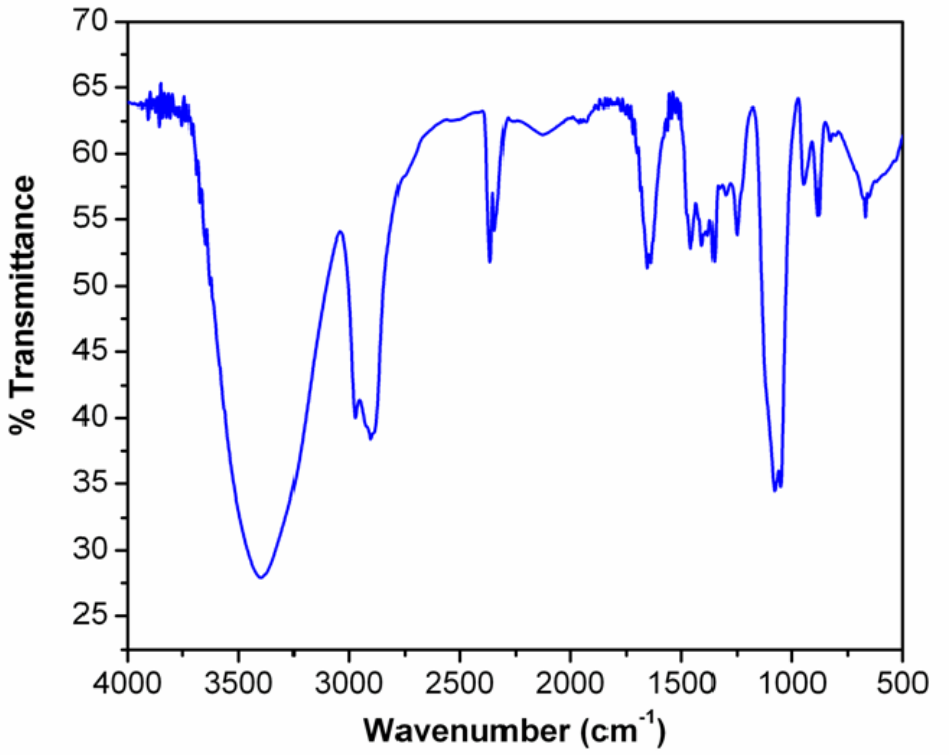

Figure S3. FTIR spectra of solvothermally synthesized CQDs.

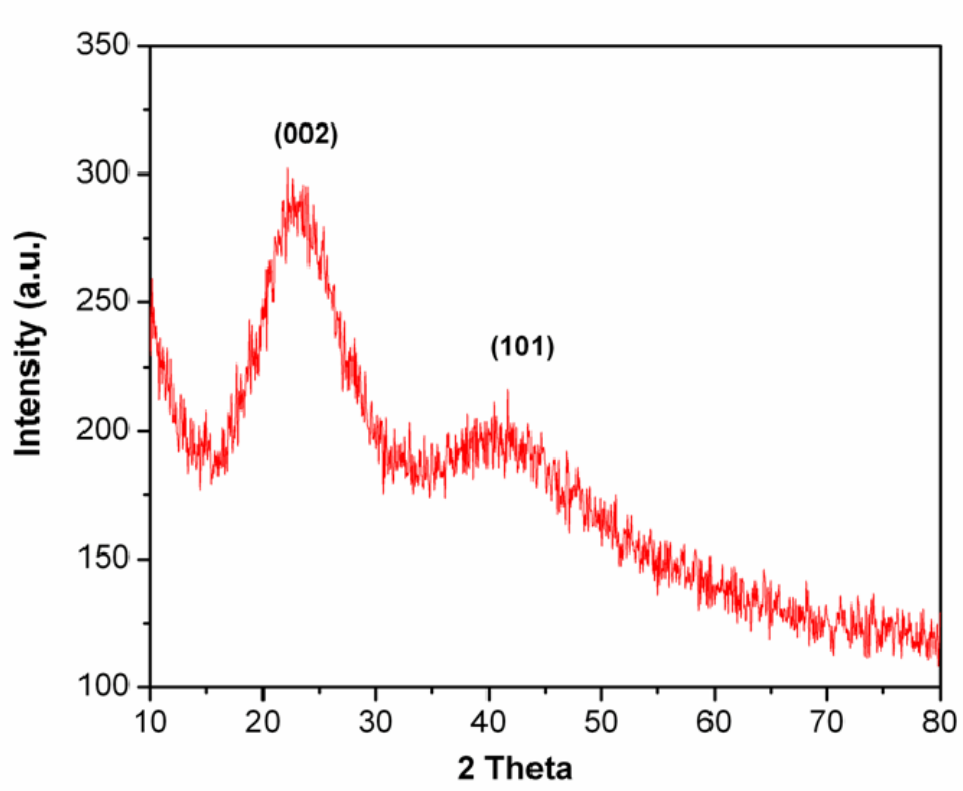

Figure S4. XRD pattern of solvothermally synthesized CQDs.

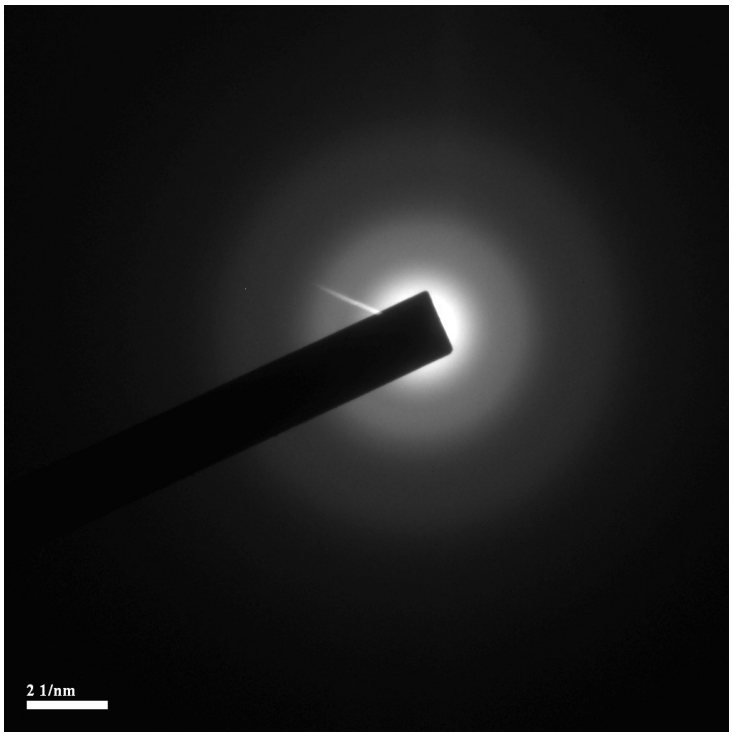

Figure S5. SAED pattern of solvothermally synthesized CQDs.

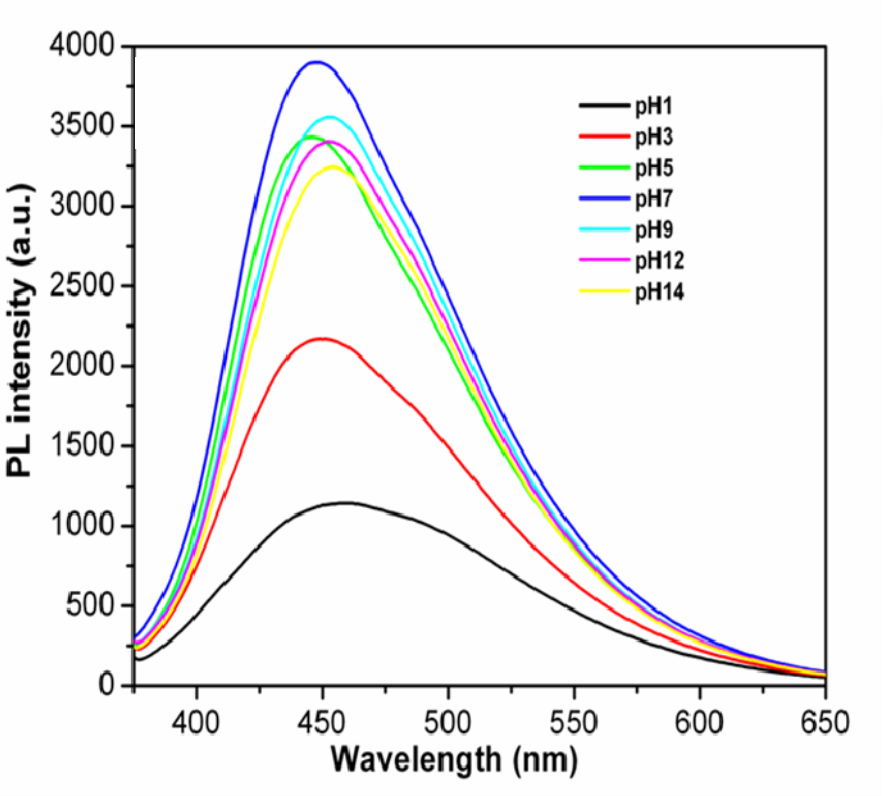

Figure S6. Change in PL intensity as a function of pH.

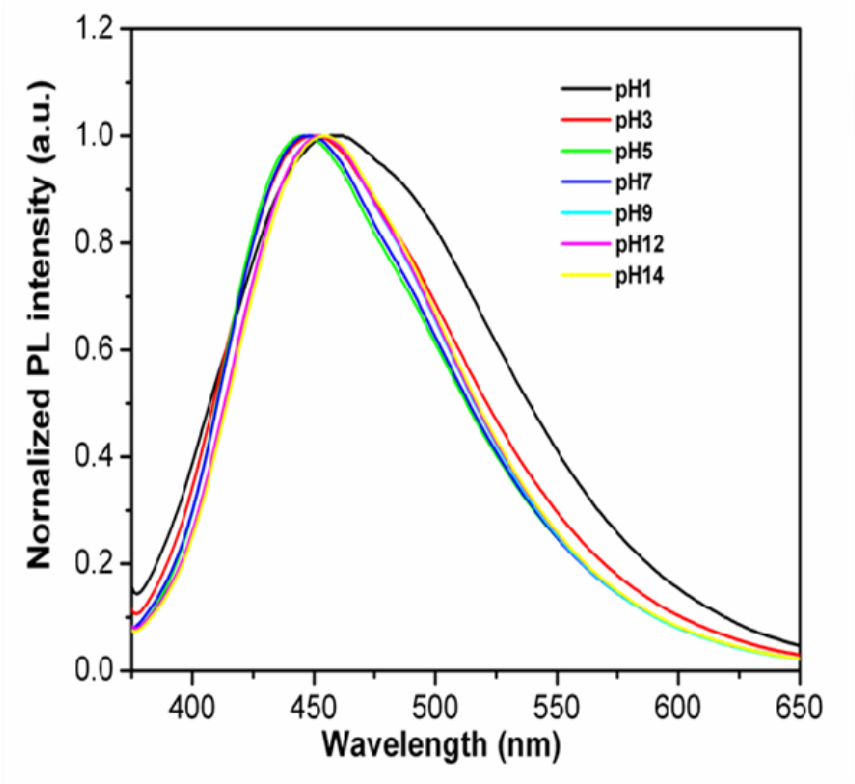

Figure S7. Normalized PL intensity at different pH.

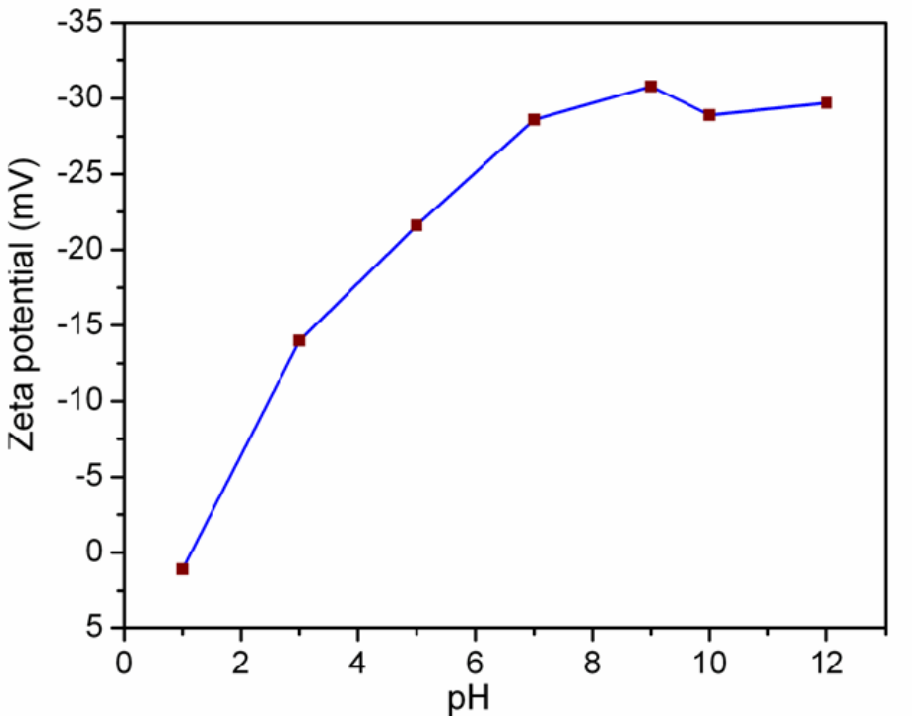

Figure S8. Measurements of zeta potentials of solvothermally synthesized CQDs as a function of pH.

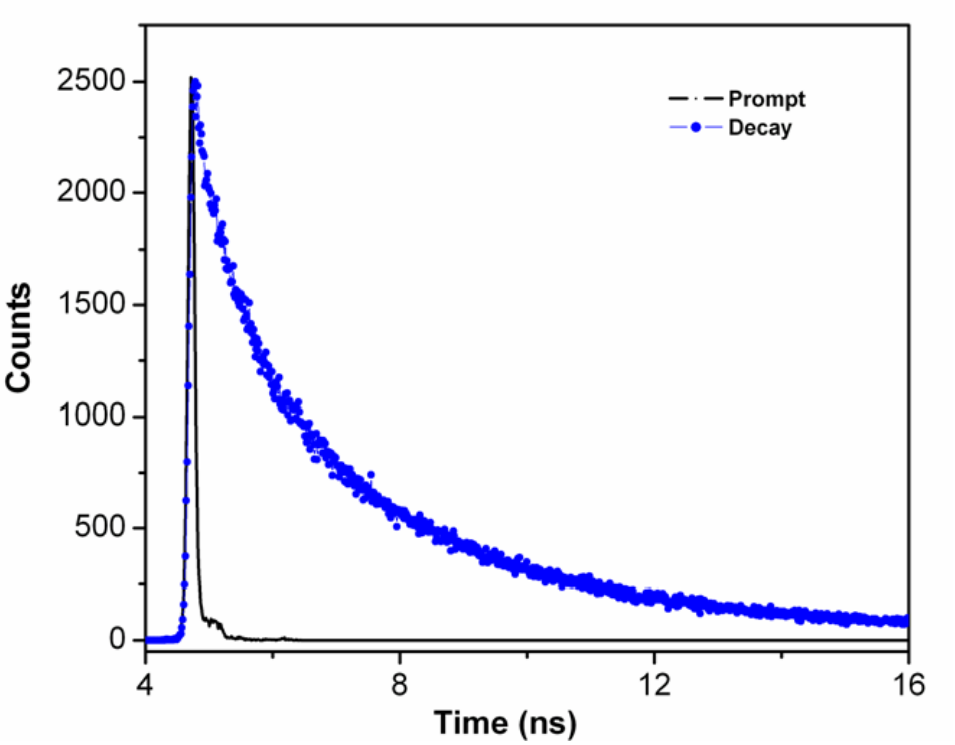

Figure S9. TCSPC lifetime profile of solvothermally synthesized CQDs.

Table1. Tabular representation of TCSPC lifetime measurements

Substance	**a_1**	**τ_1 (ns)**	**a_2**	**τ_2 (ns)**	**a_3**	**τ_3 (ns)**	**$<\tau>$ (ns)**	**χ^2**
CQD (solvothermal)	0.4294	2.17	0.4460	0.3	0.1246	6.56	1.88	0.97

Average lifetime was calculated with the help of the following equation:

$$\tau_{av} = a_1\tau_1 + a_2\tau_2 + a_3\tau_3$$

where τ_1, τ_2, τ_3 were the first, second and third component of the decay time of GCQD and a_1, a_2, a_3 were the corresponding relative weightings of these components respectively.

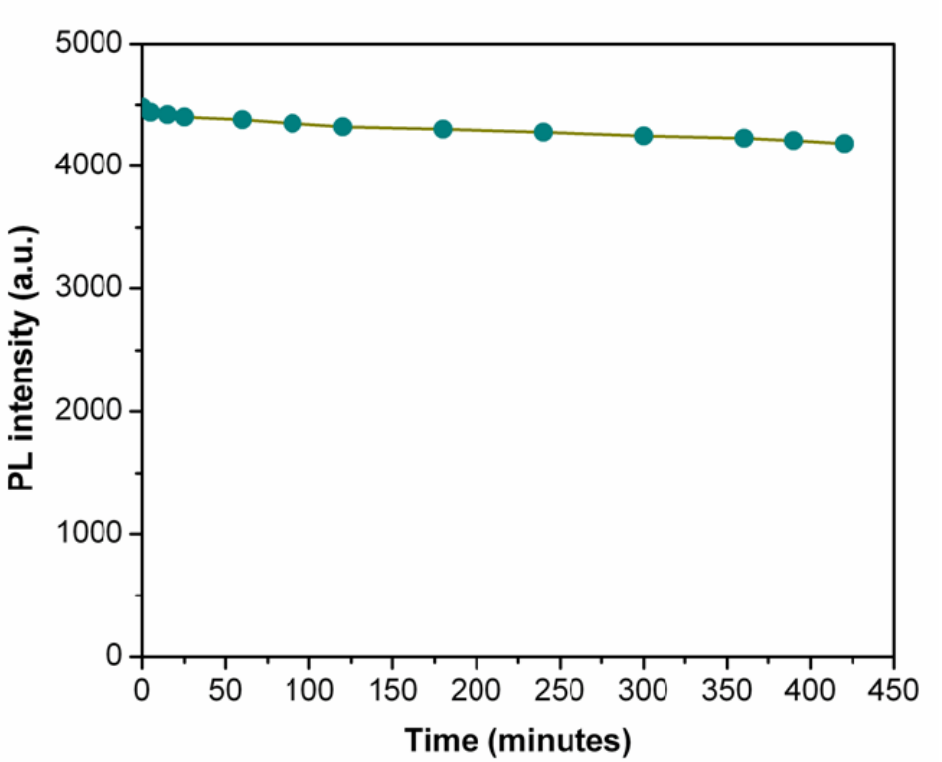

Figure S10. Plot of PL intensity against time (minutes) showing photostability profile of solvothermally synthesized CQDs.

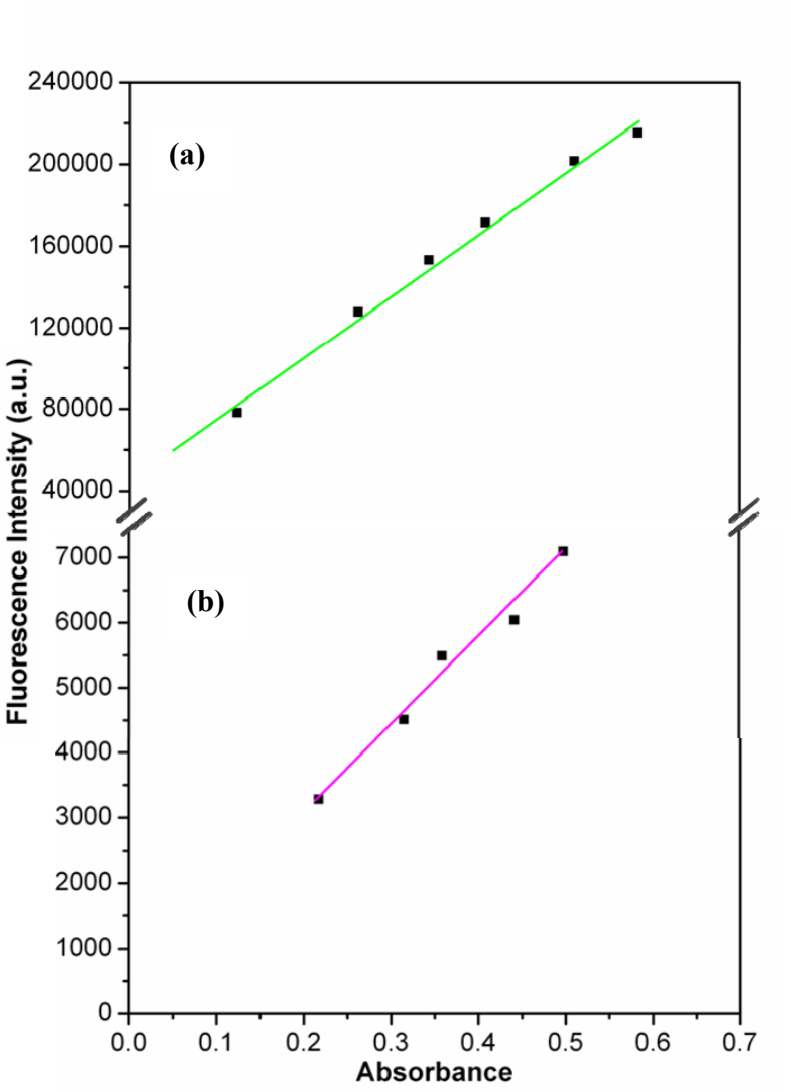

Figure S11. Plot of integrated PL intensity of (a) quinine sulphate and (b) CQDs against different absorbances.

Quantum yield of CQD was calculated by measuring the integrated PL intensity in water medium (refractive index η= 1.33) against quinine sulphate in 0.1(M) H_2SO_4 (refractive index η= 1.33) as the standard of known quantum yield 54% using the following formula.

$$\Phi_C = \Phi_{QS} \times (I_C/I_{QS}) \times (\eta_C^2/\eta_{QS}^2)$$

Where Φ, I and η represented the quantum yield, slope of integrated PL intensity and refractive index respectively. The suffix QS and C denoted quinine sulphate and CQDs respectively.

Substrate	**Slope of integrated PL intensity**	**Refractive index**	**Quantum yield**
Quinine sulphate	403862.31	1.33	54%
CQD (solvothermal)	14614.08	1.33	x

$$\Phi_C (x) = 54 \times (14614.08/403862.31) \times (1.33^2/1.33^2)\ \%$$

$$= \mathbf{1.954\%.}$$