One Step Synthesis of C-dots by Microwave Mediated Caramelization of Poly(ethylene glycol) †

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Electronic Supplementary Information (ESI)

Experimental section

Synthesis of C-dots:

PEG 200 (Merck, India Pvt. Ltd.) and ultrapure MilliQ water (>18MΩ cm) were mixed in 3:1 ratio (v/v) in a glass vial yielding a transparent solution. The solution was then heated in 900 W domestic microwave oven (Onida) for varying time period, which resulted in the formation of C–dots indicated by the development of golden yellow colour.

Synthesis with higher molecular weight PEG resulted in two kinds of observations. The polymers with molecular weight less than 800 Da (which were liquid at room temperature) resulted in the formation of C-dots as was observed using UV-vis and fluorescence measurements. On the other hand, the polymers with higher molecular weight, which were solid at room temperature, were needed to be dissolved in water before microwave irradiation. Prolonged irradiation did not result in the formation of any fluorescent species, whereas, a waxy solid could be observed to have remained in the container following evaporation of water.

Characterisation:

Transmission electron microscopy (TEM) measurements were performed in JEOL 2100 UHR-TEM instrument at a maximum accelerating voltage of 200 kV. 5 µL of sample was drop-cast on carbon-coated copper grids and subsequently air-dried before TEM analysis. UV–Vis and fluorescence spectra were recorded with a Perkin Elmer Lambda 25 and a Perkin Elmer LS 55 instruments respectively. Time–resolved fluorescence intensity decay of the C–dots was recorded using a Life Spec II spectrofluorimeter (Edinburgh Instrument). The sample was excited by 375 nm laser, and the decay was measured in a time scale of 0.024410 ns/channel. The decay curves were analyzed by FAST software, provided by Edinburgh Instrument along with the fluorescence instrument. The generated curve for intensity decay was fitted in the function

$$I(t) = \sum_i \alpha_i \exp\left(\frac{-t}{\tau_i}\right)$$

Where α_i is the initial intensity of the decay component i, having a life time of τ_i .

The average lifetimes of C-dots were calculated using the following equation

$$\langle \tau \rangle = \frac{\sum_i \alpha_i \tau_i^2}{\sum_i \alpha_i \tau_i}$$

X-ray diffraction (XRD) patterns were recorded with a Brucker D8 Advanced X-ray diffraction measurement system, with Cu Kα source (λ =1.54 Å).

Electrophoretic Separation of C-dots:

The prepared C–dots were loaded to a 20% poly(acrylamide) gel containing SDS as the denaturing agent and electrophoretic separation was carried out at 200 V at proper cooling

conditions in a vertical gel electrophoresis unit (Hoefer®). After electrophoresis, the gel was visualized by illumination using a UV trans-illuminator (λ = 312 nm).

Cell Culture and viability assay:

HT 29 cells (human colon adenocarcinoma) was procured from National Center for Cell Sciences (NCCS), Pune, India and were cultured in Dulbecco's modified Eagle's medium supplemented with penicillin (50 units mL^{-1}), streptomycin (50 mg mL^{-1}), and 10% (v/v) fetal bovine serum. Cells were maintained in 5% CO_2 humidified incubator at 37 ^{o}C.

For the cell viability assay, cells were seeded (10^4 cells/well) into a 96-well microplate and grown overnight. After treating with varying concentrations of the C-dots for 24 h, 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) (Sigma-Aldrich, USA) based cell proliferation assay was carried out according to the manufacturer's protocol to determine the percentage of viable cells based on the mitochondrial activity of the cells. The percentage cell viability of the nontreated cells (control) was taken as 100%. All measurements were collected in triplicate and the values are expressed as mean ± standard deviation (SD).

For cellular labelling study, HT 29 cells were seeded in 35 mm cell culture plate and grown for 48 h. It was then incubated with the C–dots in DMEM medium for 24 h. Then, the medium was removed and the cells were washed with PBS for 3 times. Finally, 1 mL of PBS was added to the plate and the cells were observed under epifluorescence microscope (Nikon eclipse Ti).

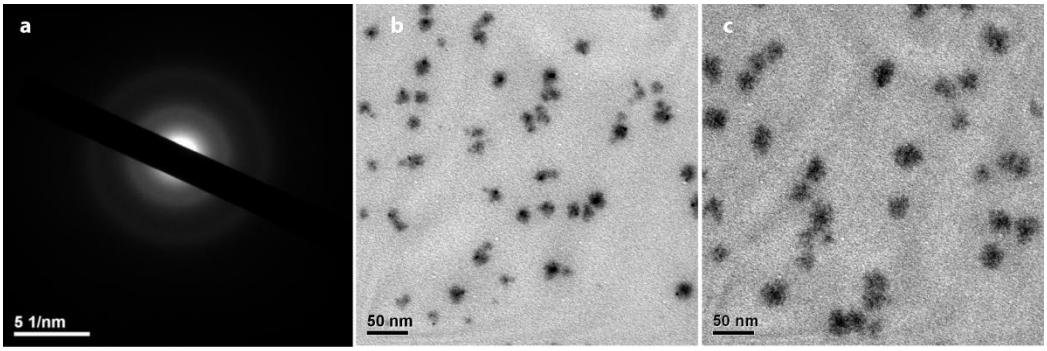

Figure S1. **a)** Selected area electron diffraction (SAED) pattern of C-dots, **b)** TEM image of C-dots synthesized after 30 min of microwave irradiation and **c)** TEM image of C-dots synthesized after 45 min of microwave irradiation.

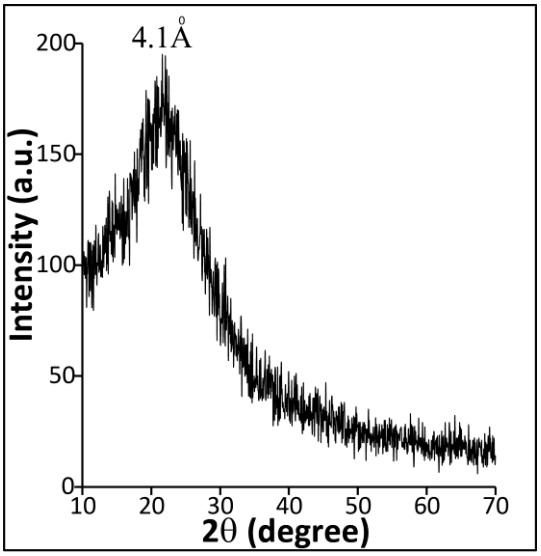

Figure S2. XRD pattern of C–dots.

Table S1. Life–time data obtained using the bi–exponential model for the C–dots.

	Value	SD	χ^2
τ_1 (ns)	3.77×10^{-9}	5.73×10^{-11}	1.01
τ_2 (ns)	1.11×10^{-8}	1.22×10^{-10}	
α_1	713.2	8.09	
α_2	317.3	8.80	

Quantum Yield Measurements

Quantum yield was measured according to established procedure[1] by using quinine sulfate in 0.10 M H_2SO_4 solution as the standard. The absorbance was measured on a Perkin Elmer LS 55 Spectrophotometer. Absolute values are calculated according to the following equation:

$$Q = Q_R \frac{m}{m_R} \frac{n^2}{n_R^2}$$

Where, Q is the quantum yield, m is the slope of the plot of integrated fluorescence intensity *vs* absorbance and n is the refractive index (taken here as 1.33, the refractive index of distilled water). The subscript R refers to the reference fluorophore, quinine sulphate solution. In order to minimize re-absorption effects, absorbance in the 1 cm quartz cuvette was kept below 0.15 at the excitation wavelength of 375 nm.

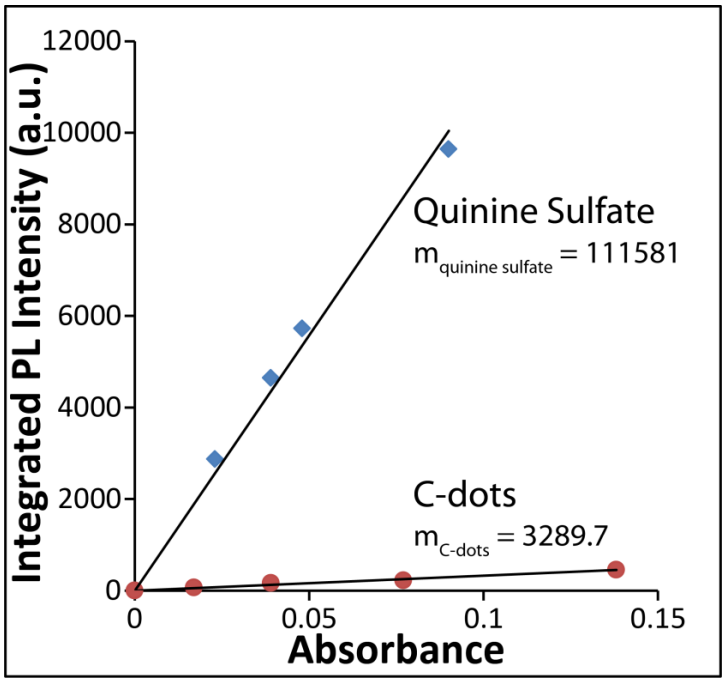

Figure S3. Integrated fuorescence intensity versus absorbance plot of C-dots and quinine sulphate.

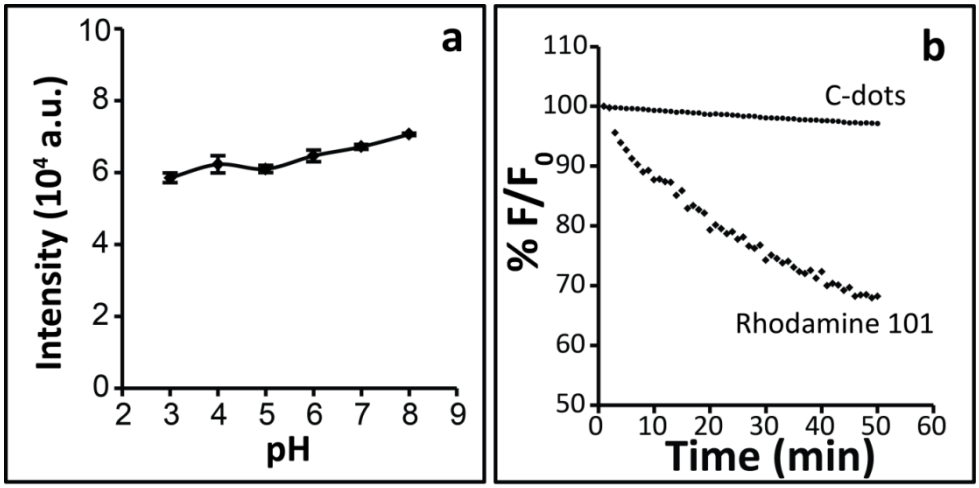

Figure S4. (a) Effect of pH on the fluorescence intensity of C–dots. All the values are average of three independent readings with ± standard deviation (SD) as error bars. (b) The effect of photoirradiation time on the fluorescence intensity of C–dots and the organic fluorophore rhodamine 101.

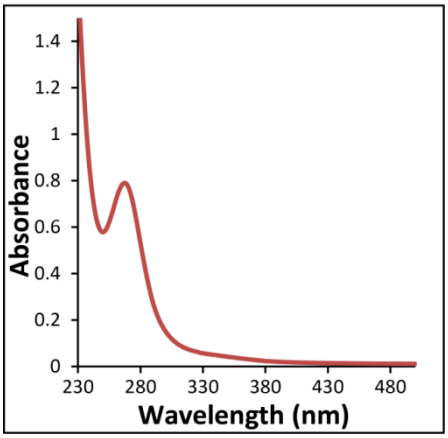

Figure S5. Absorbance spectrum of C-dots obtained from the excised fluorescent band of PAGE gel.

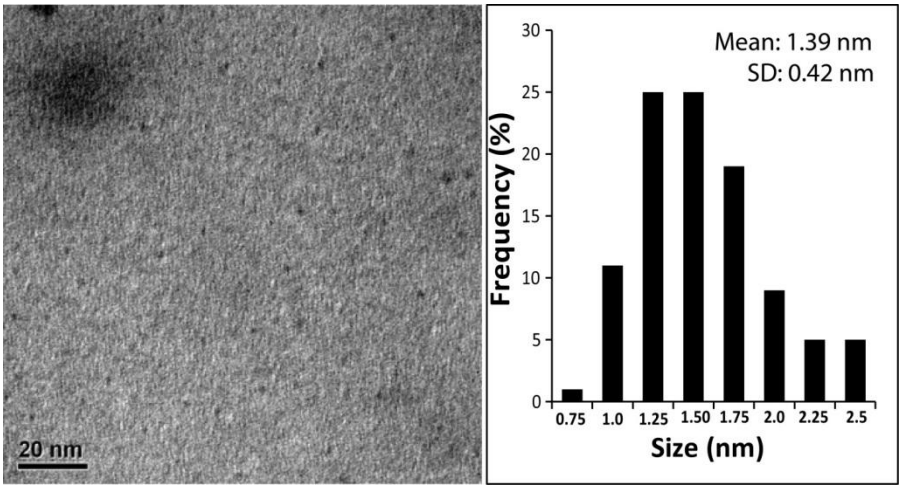

Figure S6. TEM image and corresponding size distribution of C–dots obtained from the excised fluorescent band of PAGE gel.

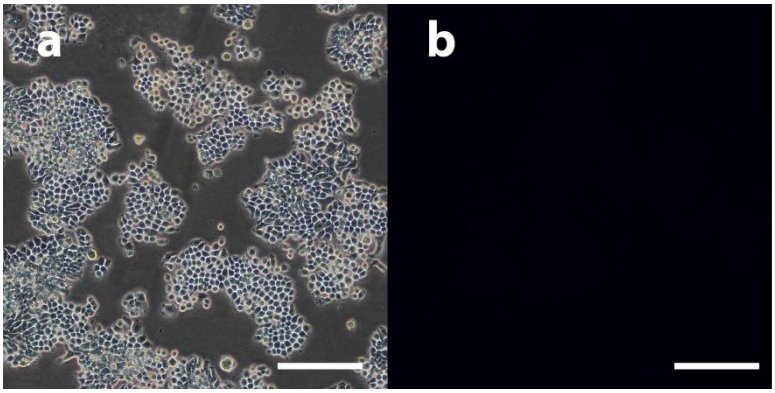

Figure S7. (a) Phase contrast and (b) Epifluorescence micrographs (under UV excitation) of HT 29 cells without any treatment with C–dots. Scale bar: 200μm.

Reference

1. J. R. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd Ed., 1999, Kluwer Academic/Plenum Publishers, New York.