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Luminescent Chitosan/Carbon Dot as an effective nano-drug carrier for neurodegenerative diseases

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Synthesis of dopamine@Chitosan/carbon dots(dopamine @CS/CD)

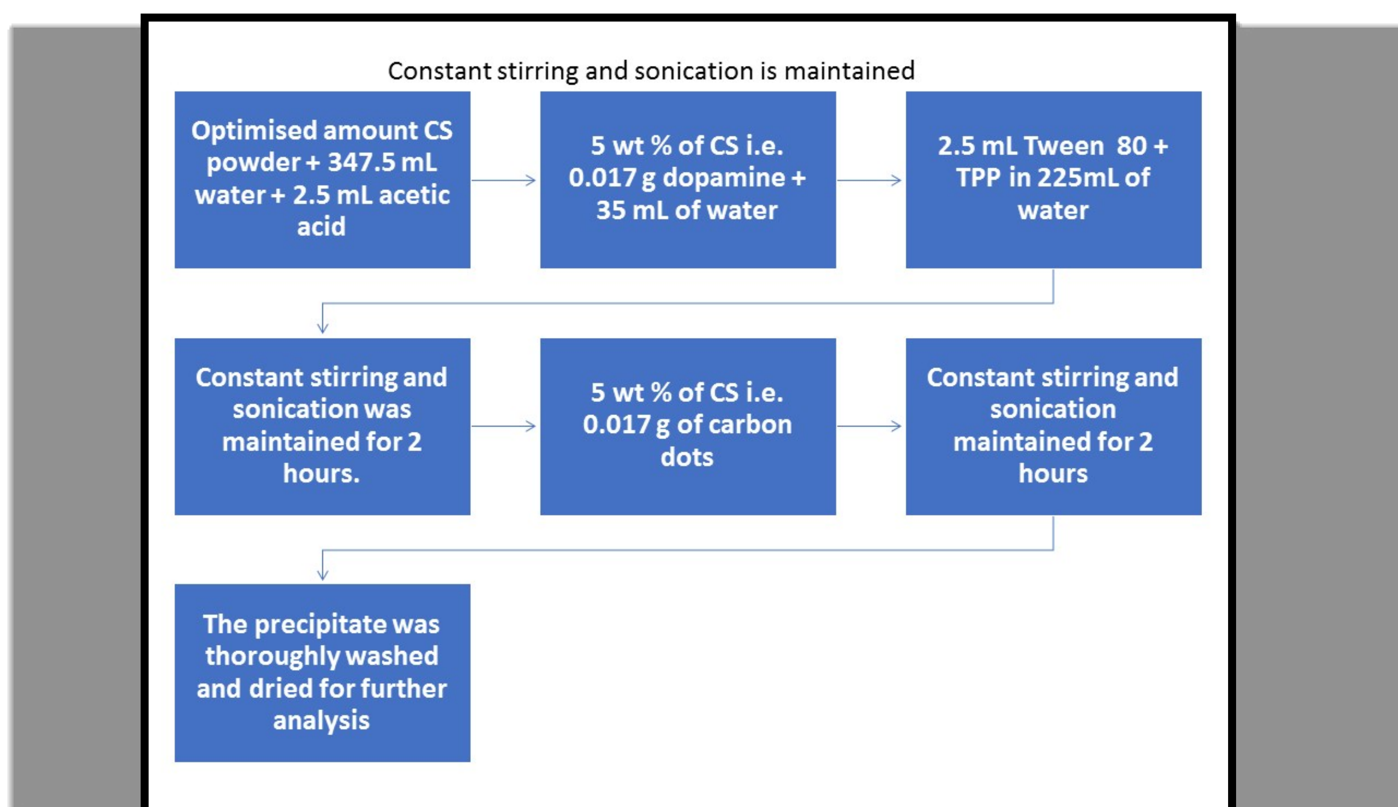

Figure S1. Flowchart for the preparation of dopamine@chitosan/carbon dots.

A detailed flowchart for the preparation of dopamine@Chitosan/Carbon dots is given in figure S1. The experiment was carried out at ambient temperature i.e. 25 ^{0}C throughout the synthesis. The composite precipitate was kept under constant sonication and was analysed and found no sign of degradation.

Thermogravimetric Analysis

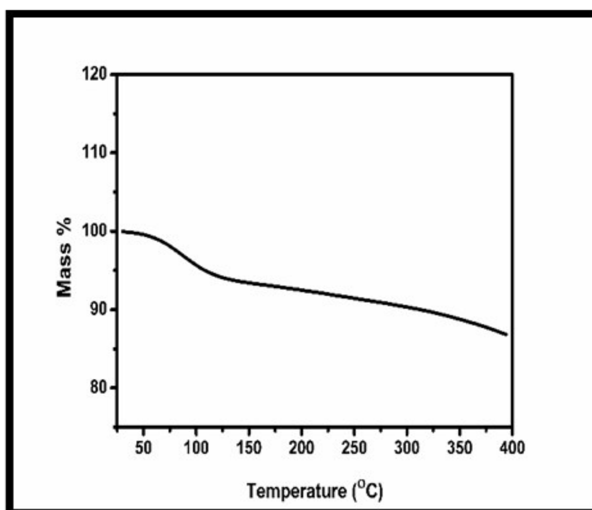

Figure S2. TGA thermogram - Mass changes with respect to temperature of as prepared carbon dots

The thermal stability of the prepared carbon dots was analysed by using Thermogravimetric analysis for the temperature that ranges from 50 °C to 400 °C as seen in figure S2. Weight loss of about 13% around 100 °C shows the evaporation of water molecules. This result ensures that the material has favourable thermal stability. The TG curve further portrays that the sample is stable. This proves that no impurities are present in the sample and that the synthesis procedure adopted produces samples of high quality.

Photoluminescence

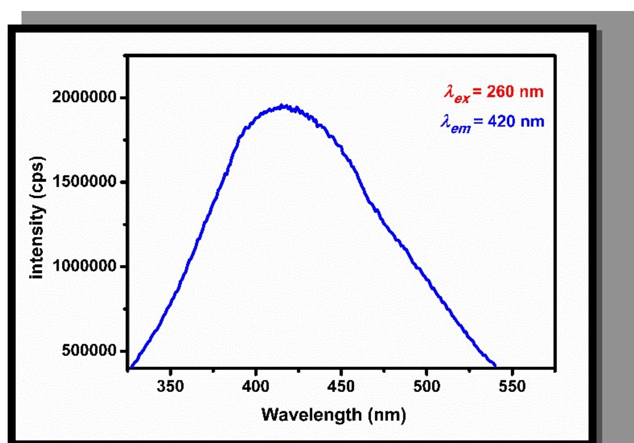

Figure S3. PL emission for excitation at 260 nm (absorption maxima)

As-prepared CDs exhibited a strong PL emission peak at 420 nm when excited with the wavelength 260 nm as shown in figure S3. As seen in the UV spectra (figure 4A.), 260 nm is the absorption maxima of carbon dots. Hence, the emission seen when excited at 260 nm, which is the characteristic spectra of carbon dots is displayed in figure S3.The emission spectrum at this particular region is mainly due to π-π^* transition of electronic energy levels, functionalization of carbon dots and size dependency.

Zeta Potential

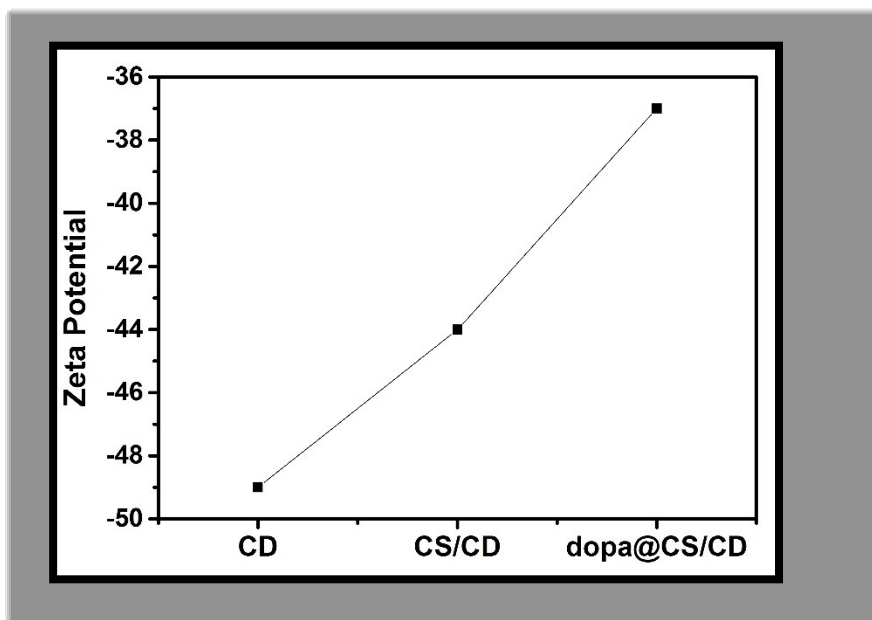

Figure S4. Zeta potential of carbon dots, chitosan/carbon dots, dopamine @ CS/CD nanocomposite.

The zeta potential values obtained for the carbon dots, chitosan carbon dots and dopamine @ CS/CD nanocomposite are seen in figure S4. As-prepared carbon dots exhibits a zeta potential of ~-49 mV. The negative zeta potential measured at pH 7 is due to the dense electron cloud around in the nitrogen doped carbon dots. Pure chitosan and dopamine have positive zeta potential. The chitosan conjugated carbon dots displayed a potential of ~-44 mV and dopamine @ chitosan/carbon dots showed a potential of ~-37 mV due to its successful conjugation with the carbon dots. The reason for this increase is the neutralization of protonated amine in the carbon dots. The zeta potential values of the drug loaded composite show their high stability which in turn evidences the encapsulation of drugs into CS/CD thus making it a suitable candidate for the drug carrier.

Drug delivery (In vitro drug release).

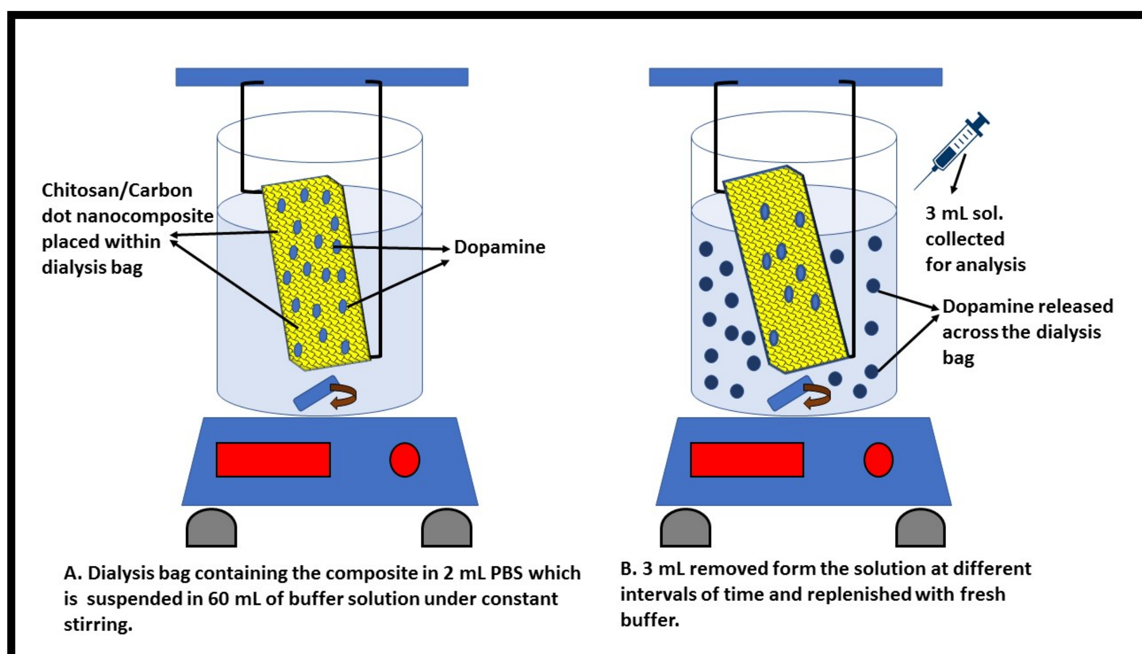

Figure S5. Schematic representation of In vitro release of the drug from the dopamine @CS/CD composite.

The scheme given in figure S5 portrays the *Invitro* drug release. 1000 mL of the buffer was prepared initially. 60 mL was transferred to another beaker. A dialysis bag containing the composite in 2 mL PBS was suspended freely as shown in the figure. This beaker was kept under constant stirring throughout the experiment. 3 mL of the buffer was removed from the 60 mL solution at different intervals of time (i.e. 1, 2, 3, 4, 6, 8 etc…) Immediately after removal, the solution was replenished with previously prepared fresh buffer. Throughout the experiment ambient temperature (i.e. 25°C) was maintained.

Encapsulation efficiency and Drug loading efficiency

The quantification of dopamine release (concentration of the drug) is directly calculated from absorption maxima of UV analysis. This concentration is denoted as the cumulative release which is shown in figures 8 A & B of the paper.

$$\text{Encapsulation Efficiency} = \frac{(Total\ amount\ of\ drug - Amount\ of\ free\ drug)}{Total\ amount\ of\ drug} \text{ X } 100 \quad \text{(E1)}$$

$$\text{Drug Loaded Efficiency} = \frac{(Total\ amount\ of\ drug - Amount\ of\ free\ drug)}{Weight\ of\ nanoparticle} \text{ X } 100 \quad \text{(E2)}$$

Cytotoxicity of IC-21 cell line

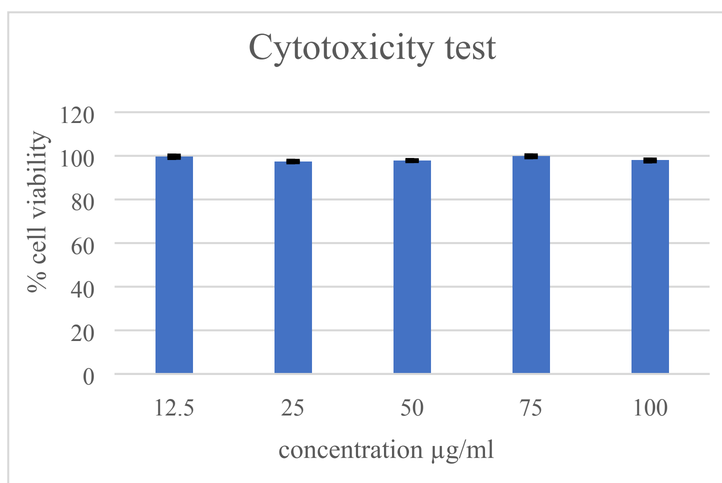

Figure S6. Plot of % viability vs sample concentration for dopamine@CS/CD towards IC-21 cell lines. Values are expressed as Mean±S.D. of 3 independent experiments.

IC-21 cell line derived from C57BL/6 peritoneal macrophages - SV40 transformed strain of a laboratory mouse and cultured in ATCC formulated RPMI-1640 medium was used to study the cell viability of the nanocomposite towards a macrophagic cell line. 1x10^{4} cells/well were seeded in a 96 well plate and allowed to reach confluency. At the start of the experiment the cells were starved in 0.5% FBS containing medium. Alamar blue reagent was used as the indicator to measure the viability of the cells. The cell medium was then treated with dopamine encapsulated chitosan/carbon dot nanocomposite at different concentrations for 24 h. Viable cells were determined by measuring the absorbance at 570 and 600 nm. The

percentage of difference in reduction between treated and control cells in cytotoxicity were calculated. The cells were found to have a viability of 97 % as seen from figure S6 and hence non-toxic in a biological environment.