



Presence of Amorphous Carbon Nanoparticles in Food Caramels

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We report the finding of the presence of carbon nanoparticles (CNPs) in different carbohydrate based food caramels, viz. bread, *jaggery*, sugar caramel, corn flakes and biscuits, where the preparation involves heating of the starting material. The CNPs were amorphous in nature; the particles were spherical having sizes in the range of 4–30 nm, depending upon the source of extraction. The results also indicated that particles formed at higher temperature were smaller than those formed at lower temperature. Excitation tuneable photoluminescence was observed for all the samples with quantum yield (QY) 1.2, 0.55 and 0.63%, for CNPs from bread, *jaggery* and sugar caramels respectively. The present discovery suggests potential usefulness of CNPs for various biological applications, as the sources of extraction are regular food items, some of which have been consumed by humans for centuries, and thus they can be considered as safe.

The use as well as presence of nanoparticles (NPs) in food is a hotly debated area, owing to their short and long term effects on human health and the environment^{1–4}. The promise of targeted and/or sustained release of drug, food colourants and flavours, while incorporated with NP, makes the pursuit of understanding of their functioning and fate a worthy exercise^{5–7}. Although, substantial development in the engineering of consumable NPs^{5–7} and their effects *in vitro* and *in vivo* have taken place⁸; few biodegradable NPs have entered clinical trials and have been marketed^{9–10}. While NP formulations for topical applications are accepted by majority of population¹¹, the idea of the consumption of these particles, either for curing a disease or for having nutritional or flavouring benefit, creates an alarm for the public. The reason behind this seems to be their potential effect on human health following consumption, which has received little attention; and the lack of awareness, which has raised concerns regarding the safety of nanomaterials in biological and food applications^{1–4}. A way around this problem, could originate out of direct derivation of nanomaterials from food products, especially from traditional food items. These materials could be considered safe for biological applications when there is no known toxicity and thus may possibly alleviate the misapprehension that all NPs are toxic.

History of nanotechnology is replete with examples of use of nanomaterials dating back to millennia^{12–14}. The dye used in colouring hair to black, during the Greco-Roman period, is now known to have been consisted of PbS nanocrystals (NCs)¹². Romans exhibited their mastery in technology in the Lycurgus cup by harnessing the optical properties of gold (Au) NPs¹³. The extraordinary mechanical strength and a sharp cutting edge in Damascus sabre have recently been attributed to the presence of carbon nanotubes (CNTs) and cementite nanowires¹⁴. In all the cases mentioned above, while the technology based on nanomaterials were known to different civilizations, the nanoscale nature of their functional constituents have only been revealed recently. The ‘nano’ dimensionality is not only confined to engineered materials or technology; nature also creates NPs or nanostructures which are present as functional components in an organism; either in the form of enzymes which catalyze most of the biological reactions or as ribosomes which act as the sites for protein synthesis. It was the invention of sophisticated microscopic and analytical techniques which has led to the discovery of these nanostructures. In this regard, the idea of searching for nanomaterials within regular food items cannot be inexplicit. This motivated us to search for NPs in food items, which can potentially be used for biological application, where the concern of the origin and toxicity of the nanomaterials can easily be waived off. Herein, we report the presence of carbon nanoparticles (CNPs) in regular carbohydrate based food caramels, such as in bread, *jaggery*, corn flakes and biscuits. The CNPs have been found to be present in those samples, where the preparation of food mainly involves heating of the starting ingredients in absence of water, leading to formation of caramels. Arguably; this discovery revealed that human consumption of nanomaterials in the form of food caramels has its history possibly from the period when human for the first time started eating bread.

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Carbon dots (C-dots), which are CNPs below 10 nm are emerging as viable alternatives to semiconductor quantum dots (Qdots) owing to their important photoluminescent properties and lack of any known cytotoxicity^{15–17}. The wavelength-tuneable emission properties have made them promising candidates as new ‘nanolights’¹⁷. The optimism has led to increased recent interests in developing methods for their syntheses, involving both top-down and bottom-up approaches¹⁷. Incidentally, there are also efforts to understand and tune their optical properties, based on the surface functional groups^{18–20}. We have recently observed that caramelization of poly (ethylene glycol) under microwave irradiation constitutes formation of biocompatible C-dots¹⁵. This prompted us to analyse the components of different commercial and homemade caramel containing food items for the presence of CNPs. Amazingly, we found that the light to dark brown coloured caramels present in carbohydrate containing foods such as bread, *jaggery*, corn flakes and biscuit consist of amorphous CNPs, which were similar to those obtained from caramel produced upon heating of commercial sugar. The discovery of the prominent presence of CNPs in regular food items provides simpler and safer sources *i.e.* daily food items, where the extracted CNPs can be directly used for biological applications. Further, our observations of the hydrophilicity of the surface functional groups of the CNPs, containing carboxylic and alcoholic groups which provide easier alternatives for their conjugation with different therapeutics, could further make them preferred fluorescent candidates for biological applications^{15–17}.

Results

The CNPs isolated from the outer brown part of the bread bun and the caramels obtained from commercially purchased sugar (following caramelization) and *jaggery* were analysed by UV-vis and fluorescence spectroscopy. The UV-vis spectrum of each of the dispersions consisted of a peak (marked with asterisk) and a shoulder (marked with arrow) between 240 nm and 400 nm and is shown in Figure 1. In addition, there is the presence of a strong background till 540 nm. The peaks and the background extinction are known to occur for CNPs and they are consistent with the literature reports¹⁷. The exact assignment of the peaks is still not known and hence the difference among the individual samples could not be explained. CNPs were also extracted from commercially procured biscuits and corn flakes, the spectroscopic data for which are shown in Figure S1 (*supplementary information*).

Photographs of the original samples (bread, *jaggery* and sugar) which served as the preparatory ingredients are shown in Figures 2a, 2b and 2c. The photoluminescence spectra corresponding to the above dispersions (and other samples) are shown in Figures 2d–2i (and Figure S1). The pictures of the dispersions in the presence of white light and UV light are represented in Figures 2j–2o. Under normal white light the dispersions have the characteristic caramel colour, whereas, under UV light ($\lambda_{\text{ex}}=365$ nm) it showed blue luminescence. All of the dispersions exhibited excitation dependent emission spectra as shown in Figures 2g–2i, which were similar to C-dots reported previously¹⁷. It was also observed that with increase in the wavelength of excitation from 325 to 375 nm the luminescence intensity increased, the maximum emission intensity being observed for the excitation wavelength of 375 nm, whereas, further increase of the excitation wavelength resulted in the decrease of emission intensity. Additionally, along with decrease in the fluorescence intensity with increasing excitation wavelength the emission maxima showed red-shift, displaying the property of excitation tuneable emission. The excitation dependent emission is an intrinsic property of CNPs, which has been widely reported by several research groups, including us^{15,17,21–26}. The quantum yields (QYs) of the CNPs obtained from different food sources were calculated using quinine sulphate as the standard²⁵. At an excitation wavelength of 365 nm, the QYs of the CNPs are

summarized in Table 1. The results indicated that these samples had QY typical of C-dots, which is on the order of 1%; with the highest being observed for samples from bread (1.2%) and that from *jaggery* had the lowest value (0.55%).

Transmission electron microscopy (TEM) images of the samples obtained from the dispersions of different caramel sources (bread, *jaggery* and sugar) are represented in Figures 3a–3c, which showed the presence of uniform spherical NPs. The particle distributions calculated from the images are shown in Figures 3d–3f. The average particle sizes as calculated from the TEM images for samples from bread, *jaggery* and sugar caramel, were determined to be 27.5 ± 6.1 , 20.3 ± 7.5 , 4.3 ± 1.5 nm respectively. Similarly, samples from corn flakes and biscuits indicated the presence of NPs having sizes of 10.5 ± 2.8 nm and 3.9 ± 1.3 nm respectively (Figure S1, *supplementary information*). The results clearly indicated that NPs were present in the dispersions extracted from bread, *jaggery*, caramel of sugar and other materials. While the sample from bread had the highest particle size, the particles from sugar caramel produced at 200°C had the lowest size and the particle sizes of the sample from *jaggery* were in between the two. In addition, caramels from sugar, produced by heating at 180°C for 10 min, had particles of size 25.8 ± 12.4 nm (Figure S2, *supplementary information*). Thermogravimetric analysis of sugar indicated decomposition starting at below 200°C with steady decrease in weight till 350°C (Figure S3, *supplementary information*). Loss of weight signifies the dehydration process of carbohydrate or formation of CO₂²⁷. Thus, the NPs could possibly be produced at a temperature even lower than 200°C. Samples from bread, *jaggery* and caramel showed broad X-ray diffraction (XRD) peak at about $2\theta = 18^\circ$ (Figure S4, *supplementary information*), with no clear signature for crystalline nature of any of the samples. The above results indicated that NPs present in the caramels of bread, *jaggery*, corn flakes, biscuits and sugar possibly consisted of amorphous carbon.

Further, ¹³C NMR (nuclear magnetic resonance) spectra of samples from bread, *jaggery* and caramel (Figures S5, S6 and S7, *supplementary information*) revealed the presence of peaks between $\delta = 60$ ppm and $\delta = 105$ ppm. The peaks clearly indicated the presence of carbohydrate unit in the extracted CNPs. There were two additional ¹³C peaks observed in case of CNPs extracted from bread ($\delta = 216.951$ ppm, $\delta = 31.778$ ppm) and sugar caramel ($\delta = 215.578$ ppm, $\delta =$

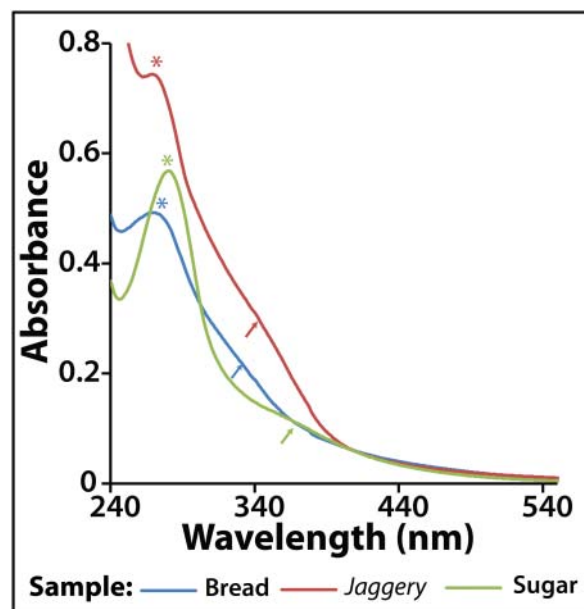


Figure 1 | UV-vis spectra of CNPs extracted from bread, *jaggery* and sugar caramel.

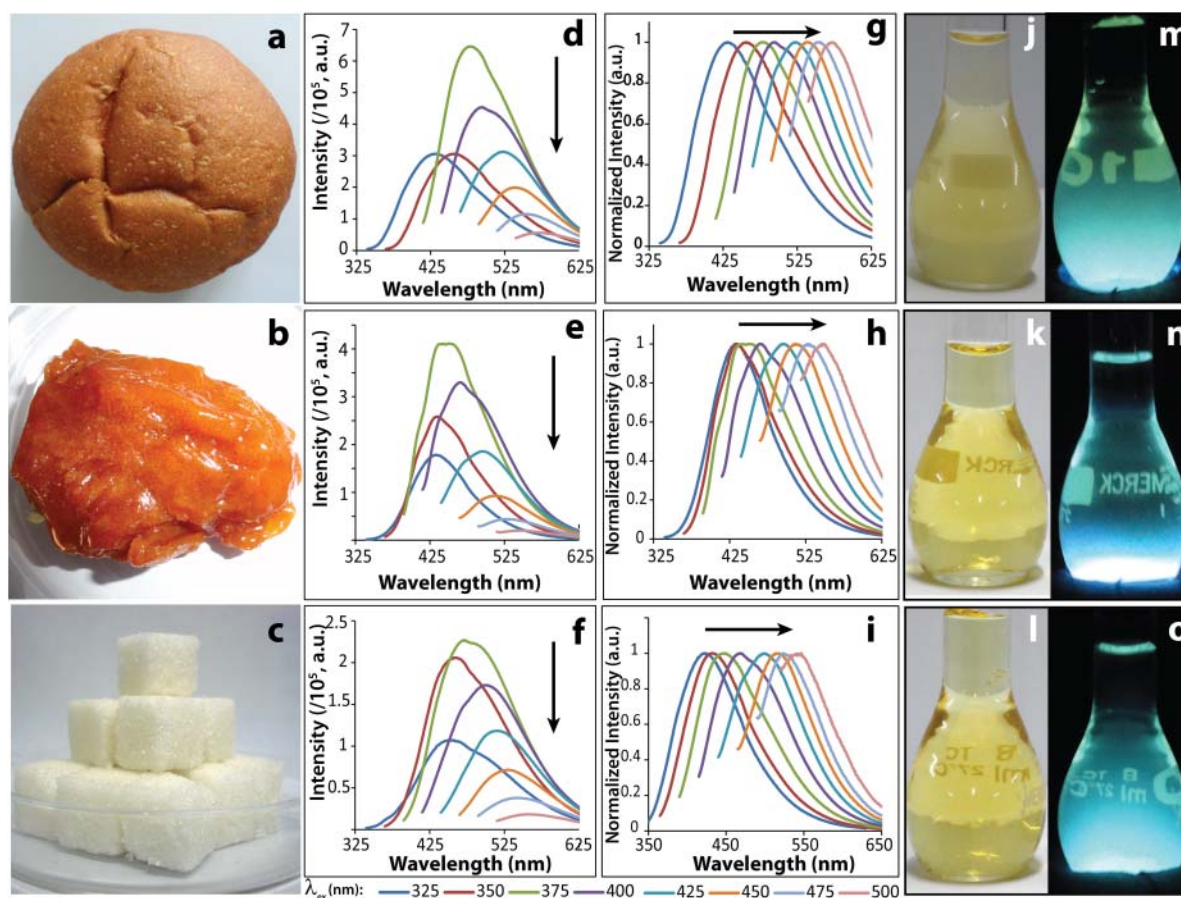


Figure 2 | (a), (b), (c) Photographs of commercial bread, *jaggery* and sugar. (d)–(i) excitation wavelength dependent emission spectra of CNPs from bread, *jaggery* and sugar caramel. (j)–(l) photographs of dispersions of CNPs from bread, *jaggery* and sugar caramel observed under white light and (m)–(o) the same under UV light.

30.428 ppm), depicting the presence of keto ($-C=O$) and methyl groups in the surface functional moieties.

Further, in order to probe the extent of cytotoxicity of the extracted CNPs, we performed XTT based cell viability assay at varying concentrations of CNPs. The plot of percentage viability of cells to that of varying concentration of CNPs (0.05 $\mu\text{g/mL}$ to 2.0 $\mu\text{g/mL}$) is shown in Figure S11. As is clear from the figure, no cytotoxicity was apparent even at the highest concentration of CNPs (2.0 $\mu\text{g/mL}$) used. In addition, one way ANOVA showed that the differences in the mean percentage viabilities of cells at different concentrations of CNPs extracted from *jaggery* ($F = 0.652$, $P = 0.689$) and bread ($F = 1.152$, $P = 0.384$) were not statistically significant.

Discussion

The UV-visible and fluorescence spectra of the dispersion of CNPs extracted from different food sources displayed features similar to those of C-dots synthesized chemically and thereby suggesting the presence of CNPs in the samples. The fundamental mechanism of photoluminescence of CNPs is still a major question; however, it is

thought that the presence of different surface trap sites could be one of the factors for the luminescence.^{17,22} The origin of fluorescence from the obtained dispersion could only be attributed to the presence of CNPs because the analysis of the starting material for preparation of bread did not show any significant fluorescence (Figure S8, *supplementary information*). Sugar is known to be a nonfluorescent material, but the caramel prepared upon heating sugar showed the emergence of excitation tuneable luminescence, further confirming the formation of CNPs. Additionally, it was observed that the heating temperature for preparing the caramel had significant effect on the size of NPs formed. Caramels prepared at 180°C and 200°C had the sizes of 25.8 ± 12.4 and 4.3 ± 1.5 nm respectively. This indicated that smaller particles were possibly formed at the higher temperature. In other words, the larger particle sizes of NPs obtained from bread and *jaggery* could be due to their low heating temperatures, whereas, the smaller particles sizes of NPs obtained from sugar caramel, corn flakes and biscuits could be due to higher heating temperatures. It may also be mentioned here that there could be other factors, such as the rate and duration of heating and chemical constituents of the samples, determining the sizes of the produced CNPs. The possibility of the formation of CNPs while preparing and analysing the sample in electron microscopy can be ruled out because when drop cast sample from sugar solution was observed in TEM no such particle formation was detected, even under the exposure of a 200 kV electron beam for several minutes. The images obtained at different time of irradiation, of the sample from sugar solution, in the electron beam of TEM are shown in Figure S9 (*supplementary information*). It is worth noting that similar extraction process was also used for determining the presence of CNPs, if any, in the interior white part of

Table 1 | Quantum yield of CNPs extracted from different food items

CNPs extracted from	Size (nm)	QY (%)
Bread	27.5 ± 6.1	1.2
<i>Jaggery</i>	20.3 ± 7.5	0.55
Sugar caramel	4.3 ± 1.5	0.63

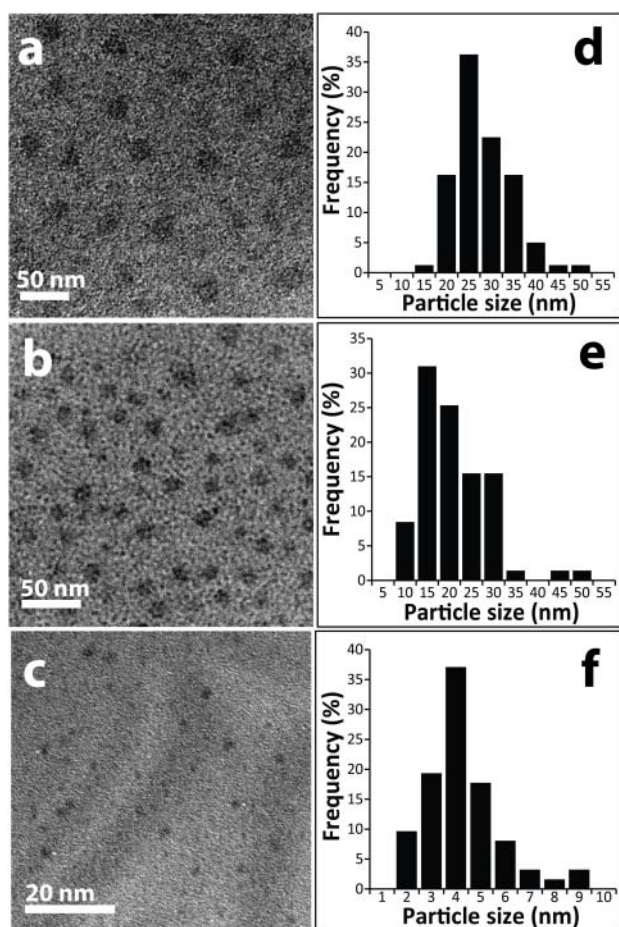


Figure 3 | (a), (b), (c) TEM images of CNPs extracted from bread, *jaggery* and sugar caramel and (d), (e), (f) corresponding particle size distributions of samples in that order.

bread. TEM analysis revealed the presence of inhomogeneous particles (Figure S10a, *supplementary information*) which could be due to the suboptimal temperature in the inner zone. The fluorescence intensity of this dispersion was significantly low compared to that obtained from the brown part of the bread (Figure S10b, *supplementary information*). The size of the CNPs produced varied from sample to sample, indicating the possibility of heating temperature as the primary factor determining their sizes. However, it was interesting to observe that for all samples the particles produced were nearly uniform and spherical. To have an idea of the amount of CNPs which can be extracted from a food source we analysed the amount of particles obtained from 1 g each of *jaggery* and the brown layer of bread. It was observed that about 3 and 2 % w/w of CNPs, in the respective samples were present. The calculation is based on the amount of the starting ingredient taken for the isolation of CNPs and the sample recovered after purification. However, the amount recovered from these materials cannot solely be attributed to CNPs as polymeric layer will always remain surrounding these particles. Isolation of nude CNPs without the polymeric layer has not been possible in the present condition; even then it can give an approximate value about the fraction of particles extracted.

Amorphous nature of NPs present in all the samples is demonstrated by the results of powder XRD data (Figure S4, *supplementary information*) as no peaks of crystalline origin was detected. The NMR studies revealed that the CNPs were coated with hydrophilic carbohydrate units. No peaks corresponding to the aromatic region was observed, which again supported the luminescence to be originating from the CNPs present in the dispersion.

In summary, our current work revealed the presence of CNPs in carbohydrate based regular food caramels from bread, *jaggery*, corn flakes and biscuit. The excitation wavelength dependent emission characteristic of the CNPs from food caramels were similar to those generated from sugar; however, the particle sizes varied indicating temperature - dependent formation of CNPs of different sizes. NMR spectroscopy revealed that the CNPs were coated with carbohydrate units. It is interesting to note that for centuries these caramels containing CNPs have been consumed by human beings with no known toxicity and thus it can be considered to have no or minimum risk on human health and may be used as a safe nanomaterial. Our finding of the presence of fluorescent CNPs in food caramels may also help their use in tracking and imaging conjugated biomolecules and drugs *in vivo*, without being imperilled.

Methods

Preparation and extraction of CNPs. Bread buns were purchased from the local market (Homa Bread, Guwahati, India) and analysed to check the presence of CNPs within it. The top brown layer of bread was carefully excised and 1 g of it was dissolved in 20 mL methanol by sonicating it at 35 kHz in a bath sonicator (Elmasonic TI-H-5 Elma, Germany) for 10 min. Following sonication, the volume of the methanol was reduced to 3 mL in a rotary evaporator before further purification. *Jaggery* (prepared from sugarcane juice) purchased from the market was heated following a traditional procedure which is as follows. Initially, *jaggery* (say 50 g) was mixed with water (about 10–15 mL) to make it sufficiently moist. The entire amount was then placed on a hot pan, which was being heated in the medium flame of a commercial gas stove. The mixture was constantly stirred using a kitchen spatula. In about 5 min, when the colour of the mixture turned dark brown, the entire amount was transferred to a pan containing a thin layer of oil and brought to room temperature. The oil layer helped in preventing aggregation of the mass and also in spreading the content over the pan. The *jaggery* caramel, which was ready for use then, was dissolved in methanol and allowed to stand for a few minutes to remove larger impurities. The sedimented particles from both the samples were removed by filtration. Centrifugation of the supernatant at 5000 rpm was performed further to remove impurities of smaller size. The yellow coloured supernatant thus obtained was further purified by column chromatography (using silica 60–120 mesh) with methanol:dichloromethane (2:3) mixture and finally dialysis (using 1 kDa membrane) was carried out to remove salts and other ions, if any. Similar procedures were followed for the extraction of CNPs from commercially purchased cornflakes and biscuits. Caramel was also prepared in laboratory by heating commercially available sugar. Sugar (*Daurala* Sugar Works, India) was taken in a glass vial and heated in an oil bath at 200°C for 10 min till the solid turned brown. The brown colored sticky mass was cooled down to room temperature and was then dissolved in methanol, followed by purification using column chromatography. The sample was then concentrated by evaporating the solvent in rotary-evaporator and then it was dissolved in water and finally dialysed before further analysis.

Characterization of CNPs. The extracted CNPs were characterized using TEM (JEOL 2100 UHR-TEM), UV-vis (Perkin Elmer Lambda 25) and fluorescence spectrophotometers (FluoroMax-4, *Jobin Yvon*). The TEM analysis was performed at an accelerating voltage of 80 kV, unless otherwise mentioned, and the sample was prepared by drop casting 5 μ L of the respective sample on a 300 mesh carbon coated copper grids and subsequent air drying before analysis. 13 C NMR (100 MHz) of the dispersion was carried out in a Varian 400 MHz FT-NMR using D_2O as the solvent. Thermogravimetric analysis (TGA) was performed in Q600 SDT Simultaneous DSC-TGA heat flow analyzer and powder XRD study was done using a Bruker D8 Advanced X-ray diffraction measurement system, with Cu K α source ($\lambda = 1.54 \text{ \AA}$). The quantum yield (QY) was calculated using quinine sulphate in 0.10 M H_2SO_4 solution as a standard, at an excitation wavelength of 365 nm, and the absorbance was kept below 0.15. The QY of the samples were determined according to equation 1.

$$QY_S = QY_R \left(\frac{m_S}{m_R} \right) \left(\frac{\eta_S}{\eta_R} \right)^2 \quad (1)$$

Where, QY is the quantum yield, m is the slope of the plot of integrated fluorescence intensity vs absorbance and η is the refractive index of the solvent. For the aqueous solutions $\eta_S/\eta_R=1$. The subscript R refers to the reference fluorophore i.e. quinine sulphate solution and S for the sample. The values obtained are given in Table S1, *supplementary information*.

In vitro cytotoxicity assays. HeLa cells were obtained 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°C. The cells were seeded in a 96 well culture plates at a density of 5000 cells/well and were allowed to grow overnight. The CNPs were then added into the wells in a concentration range of 50 ng/mL to 2 μ g/mL and incubated in a humidified incubator for 24 h at 37°C and 5% CO_2 . XTT



(Bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) (Sigma-Aldrich, USA) based cell viability assay was carried out according to the manufacturer's protocol, to determine the percentage of viable cells. The assay is based on the metabolic activity of the cells to reduce the tetrazolium salt XTT to orange coloured compounds of formazan and the intensity of the dye is proportional to the number of metabolic active cells. The percentage cell viability of the untreated cells (control) was taken as 100%. All measurements were collected in triplicate and the values are expressed as mean \pm standard error (SE). Statistical analysis for ANOVA was performed using Sigma Plot.

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Author contributions

M.P.S, A.J. and A.C. designed the experiments and analysed the data. M.P.S. and A.J. performed the experiments and A.J. and A.C. prepared the manuscript. A.P. and S.S.G. provided ideas, analyzed the data and helped in the preparation of the manuscript. All authors discussed the results.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests: The authors declare no competing financial interests.

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