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ARTICLE

Antioxidant, Catalytic Reducing and Anticancer Properties from Hydrothermally Green Synthesized Ginger Derived Carbon Nanodots

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ABSTRACT

Carbogenic carbon nanodots containing curcuminoids and 6-gingerol layers with bulk of resonating non bonded electrons were synthesized using simple and green hydrothermal method from natural herb ginger. As synthesized carobon nanodots were characterized using UV-visible and photoluminescence spectroscopy, IR, DLS and TEM analysis. The antioxidant, catalytic reducing and anticancer properties of carobon dots were studied using *ex vivo* $KMnO_4$ reduction assay, catalytic 4-nitrophenol reduction test and *in vitro* MTT assay on MCF-7 cell line, respectively. These carbogenic carbon nanoparticles shown quantum particle size of 4 nm. The green synthesized carbon dots shown excellent *in vitro* biological antioxidant and anticancer properties along with reducing nature. This study exhibited the novelty of these green synthesized bioactive carbon nanodots for tagging and coating of bioactive materials for drug vectorization, biodetection, biocompatible cell targeting and biological applications.

KEYWORDS

Ginger carbon dots, Antioxidant, Reducing, Anticancer, MCF-7 cell line.

INTRODUCTION

Carbon nanodots (CNDs), as a new member of carbon nanomaterial family, have aroused great interest because of their outstanding water solubility, high sensitivity and selectivity to target analytes, low toxicity, favourable biocompatibility and excellent photo stability [1-3]. A number of methods for the production of carbon nanodots have been reported such as hydrothermal and solvothermal technology and needs simple equipments. Due to their excellent fluorescence, carbon nanodots have made impressive strides in sensitivity and selectivity to a diverse array of salt ions, organic/biological molecules and target gases [4-8]. The development of carbon dots as nano probes is still in its infancy, but continued progress may lead to their integration into environmental and biological applications. Carbon nanodots mainly have two major categories as carbogenic and graphitic carbon nanodots. These carbon nanomaterials can be derived from natural resources, biomolecules as carbohydrates, proteins using hydrothermal, solvothermal and microwave synthetic methods. As synthesized polymeric layer structured carbon nanodots contain N, S, O hetero atoms

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with carbon as main elemental composition with sp^2 hybridization and along with conjugation and plenty of mobile electrons. Carbogenic carbon quantum dots or carbon nanodots can be derived from natural herbs and wastes and contain mainly sp^2 hybridization and conjugation of carbon atoms and or with S, N, O atoms. Carbon nanodots are conjugated systems which have sp^2 and sp^3 hybridized carbons atoms with numbers of oxygen containing groups. Carbon nanodots obtained by the hydrothermal treatment reaction contains ionization, condensation, polymerization and carbonization by bottom-up method. Such carbon dots may contain good number of mobile electrons in polymeric layers limiting in size of 2 to 8 nm and can show excellent non blinking photoluminescence and UV-visible absorption of radiations [9-17]. So these carbon nanodots can be used for coating biomaterials, nano probes, nano vectors for bio applications [18-22]. Ginger is one of the most widely used herb condiments in the world and is used as a traditional medicinal herb in eastern countries like India, China, due to its antioxidative, anti-inflammatory and anticarcinogenic properties. Curcumin, a hydrophobic polyphenol [(1E,6E)-1,7-*bis*(4-hydroxy-3-methoxyphenyl)hepta-1,6-dienne-3,5-dione], is a yellow ingredient of ginger, which exhibits many biological activities such as antibacterial, anticancer and hepatoprotective activities. Curcumin can inhibit the growth of the human cancer cells and change the cell-surface morphology and trigger pro-apoptotic factor (*e.g.*, mitochondrial damage and caspase activation) to promote cell apoptosis, with low toxicity to other cells. In addition to curcumin, 6-gingerol (a natural analog of curcumin; [5-hydroxy-1-(40-hydroxy-30-methoxyphenyl)3-decanone]) is another abundant constituent of ginger, which exhibits antimetastatic and anti-invasive pharmacological activities on cancer cells. Such active ingredients of ginger can be accommodated in quantum dot polymeric level by carbonization of ginger to carbon nanodots for use of biological activities and applications. Here in this paper we have synthesized carbogenic carbon nanodots by use of natural herb ginger. After physicochemical characterization of these carbon nanodots, their reducing nature, catalytic activities, biocompatibility, antioxidant nature and anticancer potential have been checked by various biological screening tests.

EXPERIMENTAL

All the chemicals used for synthesis of carbon nanodots and their biological screening such as NaOH, $KMnO_4$, 2,4-dinitro phenylhydrazine, $NaBH_4$, 4-nitrophenol, vitamin-C, 5-fluorouracil, MTT reagent were of A.R. grade from S.D. Fine Chem. and Merck Ltd. Cell culture medium DMEM, 10 % fetal bovine serum, Human breast cancer cell line (MCF-7) were procured from NCCS center, Pune, India. The double distilled water from Millipore system was used throughout the synthesis and testing.

Hydrothermal synthesis of carbogenic carbon nanodots from ginger: The carbogenic carbon nanodots (CNDs) were synthesized using hydrothermal green method with some modification from natural herb ginger. In brief fresh tenders of rhizomes of ginger were purchased from local market and washed with boiled water. Then the surface cover of tenders peeled and cut into small pieces. These pieces were crushed by mortar and pestle and aqueous extract was taken in appropriate volume (25 mL) in a beaker. 0.01 M NaOH (10 mL) and 15 mL double distilled water was added in to beaker and basic extract was carbonized at 200 °C for 3 h over hot plate under air atmosphere. As formed carbon residue was diluted with double distilled water to 100 mL and filtered through filter paper no. 1 then finally the filtrate was dialyzed through dialysis membrane with porosity 2 nm for 8 h with stirring. The transparent brown liquid containing carbon nano dots stored in refrigerator for further use.

Structural and morphological characterization of carbon nanodots: The structure, hybridization, morphology, particle size and types of atoms of carbon nanodots were confirmed on the basis of physico-chemical characterization on the basis of UV-visible and IR spectral analysis, TEM, DLS analysis. Systronic double beam spectrometer was used for UV-visible spectral analysis of carbon nanodots with solution conc. of 10 µg/mL in water prepared after drying carbon nanodots suspension at 100 °C with water as blank. TEM image and DLS scattering for particle size of carbon nanodots determined with original carbon nanodots aqueous suspension. IR spectra of carbon nanodots determined using KBr pallet method on Perkin Elmer series spectrometer.

Reducing catalytic activity of carbon nanodots: The reducing nature of carbon nanodots along with catalytic activity was determined by reduction of 4-nitrophenol to 4-aminophenol in presence of carbon nanodots with sodium borohydride ($NaBH_4$). The role of carbon nanodots on reduction rate was studied with UV-visible spectrophotometry. The time required for reduction in presence of carbon nanodots studied by wavelength scan spectra of 4-nitrophenol reduction to 4-aminophenol. Briefly, 2 mL of 4-nitrophenol (0.01 M) and 1 mL $NaBH_4$ (0.01 M) with 1mL water taken in cuvette and 1 mL of 10 µg/mL of carbon nanodots added to this mixture. Suddenly UV-visible spectra was recorded from 2 min after reaction up to 12 min. The online real time UV-visible scan was performed until completion of reaction of 4-nitrophenol to 4-aminophenol.

Antioxidant property of carbon nanodots by *ex vivo* $KMnO_4$ assay: The antioxidant activity of carbon nanodots were tested by *ex vivo* $KMnO_4$ reduction assay with vitamin-C as standard control antioxidant drug by UV-visible spectrometer optometric absorbance measurement. Briefly 5 mL 0.01 M $KMnO_4$ reacted with 5 mL 1 mg/mL. Carbon nanodots in a hard glass test tube sealed at open end with cotton and incubated in dark for 30 min. at 37 °C in CO_2 environment of incubator. The absorbance of bare potassium permanganate solution was determined before and after incubation with antioxidant material as OD_1 and OD_2, respectively. Same test was performed for carbon nanodots and Std. Vitamin-C as control for various concentrations of 0.12, 0.25, 0.5 and 1 mg/mL finally the percent antioxidant activity of material determined by using *ex vivo* assay formula as:

$$\text{Antioxidant activity } (\%) = \frac{OD_1 - OD_2}{OD_1} \times 100$$

Anticancer potential of carbon nanodots by *in vitro* MTT assay on MCF-7: Cancer cell cultures-MCF-7 (human breast cancer) cell lines were purchased from NCCS, Pune,

India. All cell lines were grown and maintained in suitable (DMEM-media and were grown and subcultured in medium supplemented with 10 % fetal bovine serum,1 % L-Glutamine. 1 % penicillin streptomycin antibiotic solution. All cells were trypsinated using trypsin-EDTA solution and seeded in 96-well plates.

The newly synthesized carbon nanodots were evaluated for their *in vitro* cytotoxic effects against MCF-7 (breast cancer cell line), by the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay using 5-fluorouracil (5-FU) drug as a positive control in aqueous form.

The MCF cell line was maintained in DMEM medium supplemented with 10 % fetal bovine serum. The cells were plated at a density of 1×10^5 cells per well in a 96-well plates and cultured for 24 h at 37 °C. The cells were subsequently exposed to 10 µM carbon nanodots. The plates were incubated for 48 h and cell proliferation was measured by adding 10 µL of MTT (thiazolyl blue tetrazolium bromide) dye (5 mg mL^{-1} in phosphate-buffered saline) per well. The plates were incubated for a further 4 h at 37 °C in a humidified chamber containing 5 % CO_2. Formazan crystals formed due to reduction of dye by viable cells in each well were dissolved in 200 µL DMSO and absorbance was read at 490 nm The results were compared with the standard drug inhibitors 5-fluorouracil (10 µg/mL) Lastly percent cytotoxicity of carbon nanodots was calculated by using following formula.

$$\text{Cytotoxicity (\%)} = \frac{\text{Reading of control} - \text{Reading of treated cells}}{\text{Reading of control}} \times 100$$

RESULTS AND DISCUSSION

Infrared analysis: The IR spectra of carbon nanodots shown peaks in both the regions of functional and fingerprint signals. The functional group signal region of spectra exhibited the presence of aromatic and conjugated –OH groups, diketone, aromatic conjugated system, while fingerprint region of spectral signals shown presence of aromatic –OCH_3 aromatic-H, -CH_2 stretch and presence of sp^2 hybridized carbon groups. The IR signal frequency at 3486 cm^{-1} is due to the aromatic –OH groups. Peak at 2930 cm^{-1} show presence of conjugated diketonic group. Peak at 1644 cm^{-1} may be attributed to aromatic -OCH_3. All other peaks of spectra in fingerprint area may be due to presence of conjugated –CH groups, aromatic-H, -C=O *etc*. (Fig. 1). Hence all these evidences prove the presence of curcuminoids and 6-gingerol layer of carbon nanodots. Hence these carbon nanodots are carbogenic carbon nanoparticles containing sp^2 hybridized conjugated carbon atoms containing plenty of Pi and non-bonded electrons with chain sizes in nanometer range.

UV-visible absorption spectra and photoluminescence spectra of carbon nanodots: The UV-visible spectra of carbon nanodots shows two absorption peaks at 210 nm and 315 nm and with long tailing in visible spectra. These observations clearly indicates the presence of π and non bonding electrons in carbogenic carbon nanodots. The absorption peak at 210 nm shows n to π* transition and peak at 315 is due to π to π* transition and electron radiation relaxation (Fig. 2). Hence these spectral analysis indicates the presence of conjugated

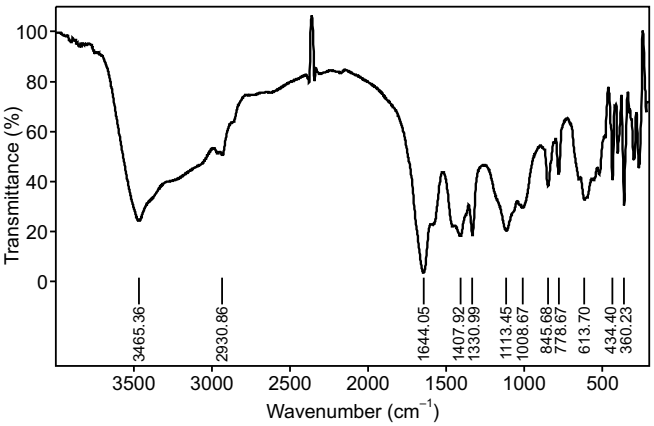

Fig. 1. IR spectra of carbogenic carbon dots prepared from ginger

carbon system with n and π-electrons probably due to curcuminoids and 6-gingerol in carbon nanodots with sp^2 hybridized carbon in conjugation. So carbon nanodots could contain aromatic conjugated natural carotenoid like diketonic molecular systems of these active ingredients of ginger.

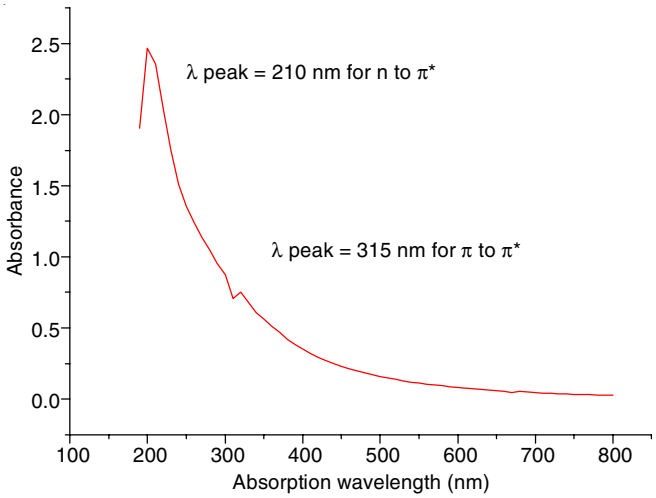

Fig. 2a. UV-visible spectra of carbogenic carbon dots prepared from ginger

Fig. 2b represents the photoluminescence spectra of carbon nanodots excited at 210 nm and shows broad fluorescence emission peaks at 470 and 495 nm corresponding to two absorption peaks in UV-visible absorption spectra hence proves presence of more number of mobile π electron and non bonded electrons on plenty of conjugated carbon atoms and few nitrogen atoms of carbon dots.

The photoluminescence spectra of carbon nanodots proves the morphology and fluorescent ability of ginger derived carbon nanodots. The emission peak at 470 nm shows that the hydrothermally synthesized carbon dots emit in visible region so as synthesized carbon nanodots have ability of strong fluorescence in visible range. Hence the possible application of carbon nanodots in biological labeling and tagging have been elaborated here by the photoluminescence spectra of carbon nanodots.

TEM image and DLS analysis: The DLS scattering spectra of carbon nanodots reveal that the average particle size of the carbon nanoparticles is 4 nm which matched with the TEM image of carbon nanodots and with size of these

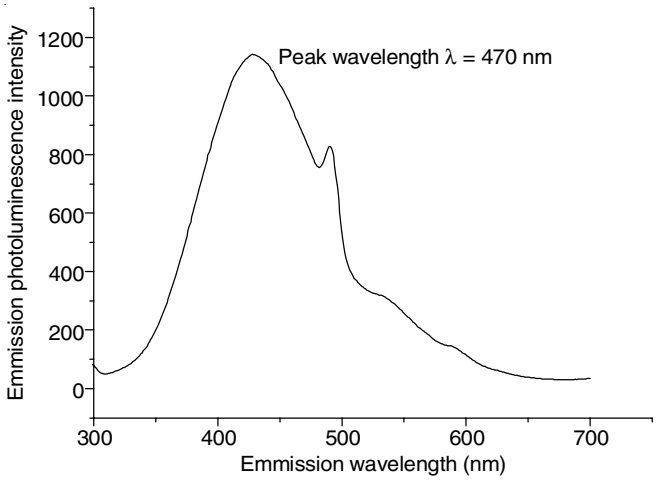

Fig. 2b. Photoluminescence spectra of carbogenic carbon dots prepared from ginger

carbon quantum nanoparticles. The sizes of carbon nanodots varies from 2 to 12 nm (Fig. 3), but maximum carbon nanodots shows size between 2 to 6 nm hence these are quantum dot carbon nanoparticles with abundance of mobile electrons responsible for light scattering in DLS and electron scattering in TEM. The TEM image of carbon nanodots proved that there is some aggregation showing amorphous nature and circular morphology of carbon nanodots material.

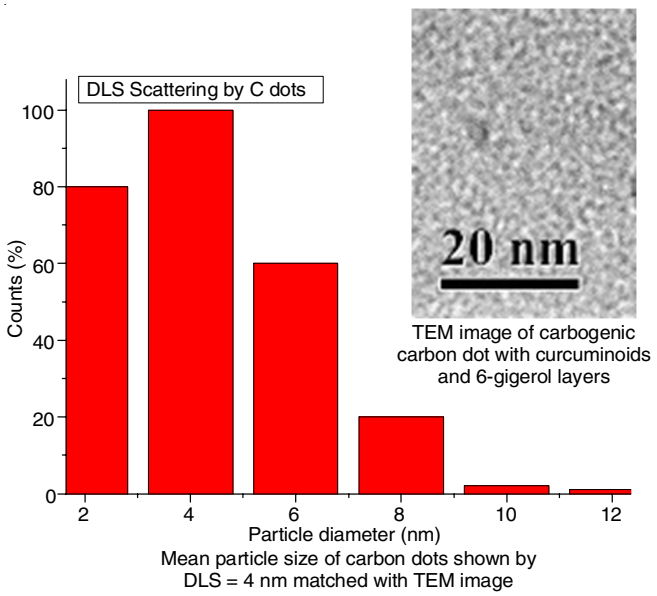

Fig. 3. DLS spectra and TEM image of carbogenic carbon dots

Reducing catalytic activity of carbon nanodots by reduction of 4-nitrophenol to 4-aminophenol: The reducing and catalytic nature of carbon nanodots tested by reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in presence of $NaBH_4$ by absorbance measurement with time lag of reaction. The initial absorption spectra of 4-nitrophenol and peak at 330 nm vanished after reduction by carbon nanodots in presence of $NaBH_4$ as hydrogen source and carbon nanodots as catalyst. After 12 min. 4-nitrophenol is totally converted to 4-aminophenol showing new absorption spectra with peak at 460 nm. UV-visible real time online reaction wavelength scan performed from 2 to 12 min until completion of reaction in cuvette. The formation of 4-aminophenol take place with shifting and dampening of peak of 4-nitrophenol and formation of new peak of 4-aminophenol in UV-visible wavelength scan spectra (Fig. 4).

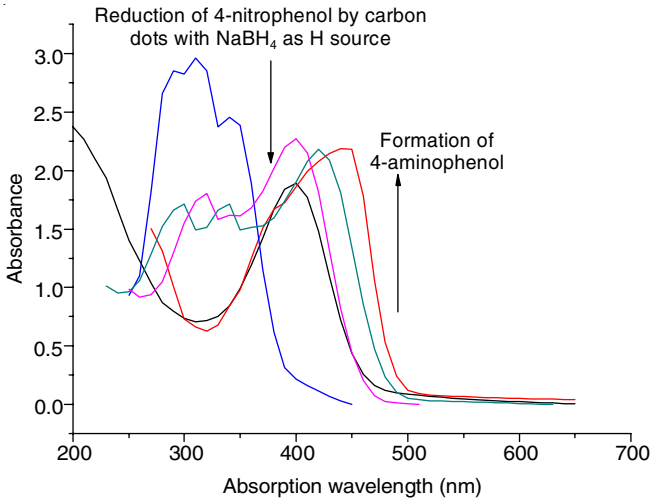

Fig. 4. Catalytic reducing activity of carbogenic carbon dots for reduction of 4-nitrophenol to 4 aminophenol

Antioxidant activity of carbon nanodots by *ex vivo* $KMnO_4$ reduction assay: Good antioxidant activity is shown by carbon nanodots derived from natural herb ginger compared with vitamin-C by *ex vivo* $KMnO_4$ assay. The antioxidant activity determined for 0.12, 0.25, 0.5 and 1 mg/mL concentrations of carbon nanodots and vitamin-C as standard control is represented in Fig. 5. The carbon nanodots shows higher antioxidant activity than vitamin-C, which increases with increase in concentration of drug. The colour of $KMnO_4$ fade after treatment of material and incubation in biological environment conditions, which elaborates the reducing as well as antioxidant nature of control vitamin-C and material carbon nanodots. EC_{50} minimal inhibition concentration value or half reducing antioxidant activity of vitamin-C and carbon nanodots determined by triplicate absorbance measurement are 0.62 and 0.48 mg/mL, respectively. So carbon nanodots are better option for antioxidant material than vitamin-C for bio applications with minimum concentration for high antioxidant activity.

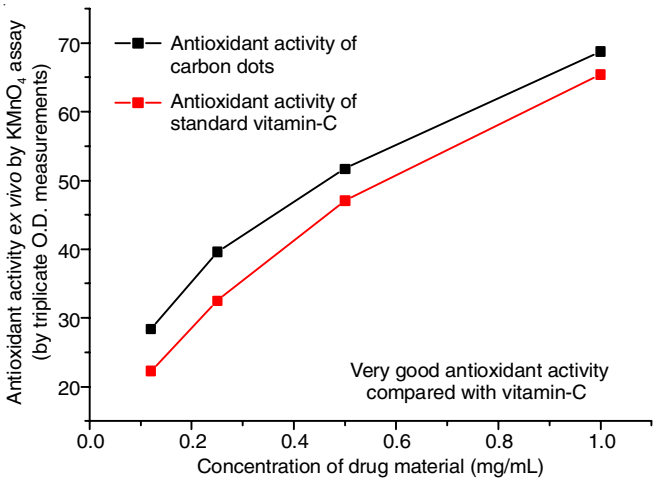

Fig. 5. Antioxidant activity of carbogenic carbon dots by *ex vivo* $KMnO_4$ assay

Anticancer potential of carbon nanodots by *in vitro* MTT assay on MCF-7 cell line: MTT assay performed on MCF-7 human breast cancer cell line for anticancer potential of carbon nanodots with 5-fluorouracil as control drug shows moderate to good activity against these cells. As carbon nanodots are reducing, antioxidant and contain free mobile electrons they can inhibit growth of MCF-7 by generation of reactive oxygen species (ROS) at acidic pH inside cells. The cell viability of MCF-7 decreased by carbon nanodots up to 64 % at concentration of 5 µg/mL in sterile phosphate buffer saline with pH = 7.4 and up to 78 % at 10 µg/mL compared with 5-fluorouracil to 15 % at 5 µg/mL and 19 % at 10 µg/mL, respectively. So the EC_{50} = 5 µg/mL shown by carbon nanodots prove that a good candidate drug for anticancer application on selected cell lines without toxicity on normal cells (Fig. 6a-c).

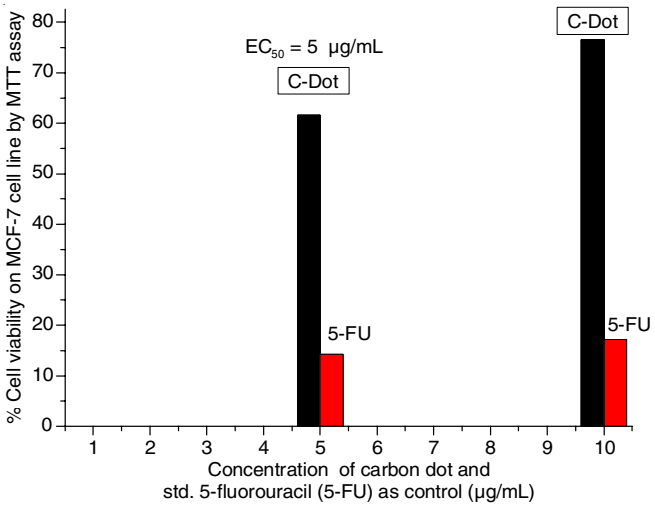

Fig. 6a. Anticancer activity of carbogenic carbon dots on MCF-7 cell line by *in vitro* MTT assay

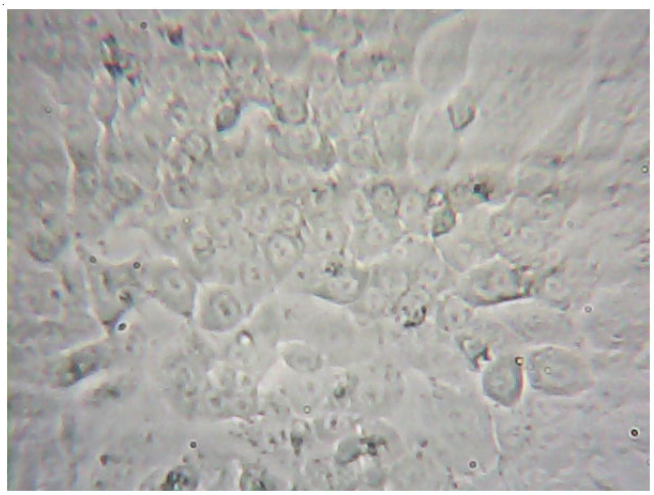

Fig. 6b. Endocytosis of C dot material and anti proliferation (cell shrinking) of MCF-7 breast cancer cell line

Conclusion

The green, hydrothermal and cost effective synthesis of carbon nanodots from natural herb ginger has been reported in this paper. The synthesized carbon nanodots had shown presence of layers of curcuminoids and 6-gingerol as drug

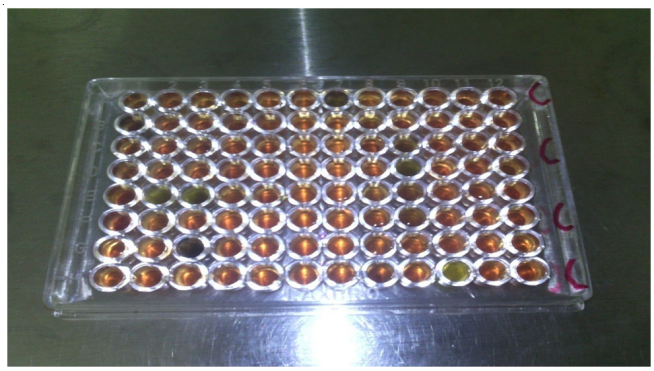

Fig. 6c. Images of MTT assay on MCF-7 Cell line

ingredients from ginger with conjugation and good number of mobile electrons. These carbon nanodots had explored reducing catalytic, antioxidant, anticancer activities. Hence these carbogenic carbon nanodots with 4 nm mean quantum size exhibited important biological activities. So these water soluble carbon nanodots derived from natural herb and in basic medium by carbonization process can be used as tagging and coating material on bioactive nanomaterials for cell vectorization or probing and for biocatalytic, antioxidant and anticancer applications. Overall the carbon nanodots derived from natural herb ginger exhibit characteristics of:

• Better reducing, catalytic, antioxidant and anticancer activities.

• Good water and phosphate buffer solubility hence these are bioavailable drug candidate.

• Good stability and low toxicity on normal cells and moderate cytotoxicity on cancer cells.

• Quantum dot size and presence of curcuminoids and or 6-gingerol natural multiactive drugs.

So these fluorescent carbon nanodots with photoluminescence emission at 470 nm can be a better options for toxic quantum dot materials as CdS and CdSe in biological applications.

REFERENCES

1. M.J. Krysmann, A. Kelarakis, P. Dallas and E.P. Giannelis, *J. Am. Chem. Soc.*, **134**, 747 (2012); https://doi.org/10.1021/ja204661r.
2. Y. Song, S. Zhu, S. Xiang, X. Zhao, J. Zhang, H. Zhang, Y. Fu and B. Yang, *Nanoscale*, **6**, 4676 (2014); https://doi.org/10.1039/c4nr00029c.
3. D. Ding, C.C. Goh, G. Feng, Z. Zhao, J. Liu, R. Liu, N. Tomczak, J. Geng, B.Z. Tang, L.G. Ng and B. Liu, *Adv. Mater.*, **25**, 6083 (2013); https://doi.org/10.1002/adma.201301938.
4. T. Lai, E. Zheng, L. Chen, X. Wang, L. Kong, C. You, Y. Ruan and X. Weng, *Nanoscale*, **5**, 8015 (2013); https://doi.org/10.1039/c3nr02014b.
5. Y. Sun, W. Cao, S. Li, S. Jin, K. Hu, L. Hu, Y. Huang, X. Gao, Y. Wu and X.J. Liang, *Sci. Rep.*, **3**, 3036 (2013).
6. C. Wu and D.T. Chiu, *Angew. Chem. Int. Ed.*, **52**, 3086 (2013); https://doi.org/10.1002/anie.201205133.
7. S. Zhu, J. Zhang, Y. Song, G. Zhang, H. Zhang and B. Yang, *Acta Chim. Sin.*, **70**, 2311 (2012); https://doi.org/10.6023/A12090690.
8. M. Sun, C.Y. Hong and C.Y. Pan, *J. Am. Chem. Soc.*, **134**, 20581 (2012); https://doi.org/10.1021/ja310236m.
9. Q. Zhu, F. Qiu, B. Zhu and X. Zhu, *RSC Adv.*, **3**, 2071 (2013); https://doi.org/10.1039/C2RA22210H.

10. Y. Hong, J.W. Lam and B.Z. Tang, *Chem. Commun.*, 4332 (2009); https://doi.org/10.1039/b904665h.
11. Y. Hong, J.W. Lam and B.Z. Tang, *Chem. Soc. Rev.*, **40**, 5361 (2011); https://doi.org/10.1039/c1cs15113d.
12. P. Mirtchev, E.J. Henderson, N. Soheilnia, C.M. Yip and G.A. Ozin, *J. Mater. Chem.*, **22**, 1265 (2012); https://doi.org/10.1039/C1JM14112K.
13. V. Gupta, N. Chaudhary, R. Srivastava, G.D. Sharma, R. Bhardwaj and S. Chand, *J. Am. Chem. Soc.*, **133**, 9960 (2011); https://doi.org/10.1021/ja2036749.
14. X. Zhang, Y. Zhang, Y. Wang, S. Kalytchuk, S.V. Kershaw, Y. Wang, P. Wang, T. Zhang, Y. Zhao, H. Zhang, T. Cui, Y. Wang, J. Zhao, W.W. Yu and A.L. Rogach, *ACS Nano*, **7**, 11234 (2013); https://doi.org/10.1021/nn405017q.
15. J. Shen, Y. Zhu, X. Yang, J. Zong, J. Zhang and C. Li, *New J. Chem.*, **36**, 97 (2012); https://doi.org/10.1039/C1NJ20658C.
16. W.-W. Liu, Y.-Q. Feng, X.-B. Yan, J.-T. Chen and Q.-J. Xue, *Adv. Funct. Mater.*, **23**, 4111 (2013); https://doi.org/10.1002/adfm.201203771.
17. J. Shen, Y. Zhu, X. Yang and C. Li, *Chem. Commun.*, **48**, 3686 (2012); https://doi.org/10.1039/c2cc00110a.
18. Z. Zhang, J. Zhang, N. Chen and L. Qu, *Energy Environ. Sci.*, **5**, 8869 (2012); https://doi.org/10.1039/c2ee22982j.
19. L. Li, G. Wu, G. Yang, J. Peng, J. Zhao and J.J. Zhu, *Nanoscale*, **5**, 4015 (2013); https://doi.org/10.1039/c3nr33849e.
20. M. Bacon, S.J. Bradley and T. Nann, *Part. Part. Syst. Charact.*, **31**, 415 (2014); https://doi.org/10.1002/ppsc.201300252.
21. X. Zhou, S. Guo and J. Zhang, *ChemPhysChem*, **14**, 2627 (2013); https://doi.org/10.1002/cphc.201300111.
22. L. Lin, M. Rong, F. Luo, D. Chen, Y. Wang and X. Chen, *Trends Analyt. Chem.*, **54**, 83 (2014); https://doi.org/10.1016/j.trac.2013.11.001.