Fabrication of multi-structure nanocarbons from carbon xerogel: A unique scaffold towards bio-imaging

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Experimental section

Materials

Resorcinol, formaldehyde, manganese acetate, nitric acid, hydrochloric acid and sodium hydroxide were purchased from Merck (India), whereas pluronic-F68 was received from Sigma Aldrich.

Synthesis of carbon xerogel

Carbon xerogel (CX) with higher surface area (>1000m²/g) was synthesized by the same procedure using our previous reported method [1]. Briefly, 2.5 g resorcinol was taken into a 100 mL of beaker, in which 20 mL of water along with 10 mL of formaldehyde were added to constitute a clear solution (resol). The pH of the solution was adjusted at 3 by addition of 0.1 (N) HCl. Simultaneously in a separate container 10 mL 20% pluronic F-68 solution was mixed with 11% of manganese acetate and the mixture was stirred vigorously to prepare a homogeneous solution. After that the resol solution was added dropwise to the metal-pluronic solution with constant stirring. Additional 110 mL water was again added to the above reaction mixture. The whole solution was stirred continuously for 12 hours in room temperature in a closed vessel and the temperature was kept at 80°C for 2 days. The polymerization was completed within these two days; the polymer was washed with distilled water for several times followed by drying in an oven at 75°C. Finally the polymer was pyrolysed in an argon atmosphere at 400°C for 1 hour and then at 850°C for 2h with the heating rate at 2°C/min.

Synthesis of carbon nanoparticles

160 mg of the above CX powder was taken in a 100 mL RB-flask fitted with a reflux condenser and mixed with 40 mL of 1:3 HNO₃: H₂O. The mixture was then refluxed at 100°C for 72 hours and allowed to cool down at ambient temperature. The resultant mixture was allowed to settle down for few hours; a yellowish brown suspension was appeared along with a black residue at the bottom of the RB-flask. The supernatant, containing carbon nanoparticles, was then separated out by centrifugation at 10000 rpm for 10 minutes leaving the black precipitate. In order to observe the characteristic change in the morphology, particle size as well as fluorescence intensity of the synthesized carbon nanoparticles, similar reactions were carried out with different concentration of nitric acid. 1:4, 1:2 and concentrated nitric acid. The colors of the supernatants were turned into pale yellow to orange depending on the concentration of the nitric acid as described above. A similar reaction was also performed for 7 days to observe the change of fluorescence intensity of the resultant nanoparticles with varying the refluxation time. During the refluxation, aliquot of the reaction mixture was taken out at an interval of 12 hours and centrifuged at 10000 rpm for 10 minutes to get the supernatant liquid. All of the above supernatant solutions were neutralized by the addition of 1(N) NaOH.

Characterizations

Fourier Transform Infrared Spectroscopy (FT-IR) was conducted by Perkin-Elmer spectrum RX-1 IR spectrophotometer. For absorption and fluorescence measurements we have used Shimadzu absorption spectrophotometer (model no: UV-1700) and Hitachi spectrofluorimeter (model no: F-7000), respectively. The phase characterization was carried out by X-ray diffractometer (XRD; model no. PW1710). Sample for XRD was prepared by the deposition of well dispersed carbon nanoparticles on glass slide and after drying the analysis was performed by using cobalt as the target material. High resolution transmission electron microscopy (HR-TEM) was carried out by using Phillips CM 200, operating at an acceleration voltage of 200 kV. The field emission scanning electron microscopy (FE-SEM) was also performed by Jeol Model JSM-6340F. For FE-SEM analysis samples were prepared by deposition followed by spin coating of the aqueous dispersion on a glass slide. Again for HR-TEM
analysis a very dilute aqueous suspension was prepared, which was then deposited on a copper grid and finally dried in air. Fluorescent microscopic analyses along with their corresponding optical images were carried out by using Carl Zeiss-Axiolab fluorescent microscope. Confocal image was acquired with 400 nm laser excitation on an Olympus laser scanning microscope. Maldi TOF-Mass spectroscopy was performed by using Applied Biosystems Voyager System with accelerating voltage at 20000V. Here dihydroxy benzoic acid (DHB) was used as the matrix material for Maldi TOF-Mass spectroscopic analysis.

Cell labeling

Microbial strain of S. Aureus was cultured in 50 mL of nutrient broth. After incubation for overnight at 37°C, 500µL of the above culture was taken and centrifuged at 3000 rpm for 2 minutes. The supernatant was then discarded. After that the bacterial pellet was resuspended in 500µL phosphate buffer saline (pH 7.4). 500 µL of MCNP–3 (0.1 mg/mL) was conjugated with the bacterial suspension by simple incubation for 4 hours under gentle shaking. The excess nanoparticles were removed by washing several times with phosphate buffer. Finally 50µL of bacterial suspension treated with MCNP–3 was taken in a cover slip, which was ready for the observation under fluorescence and confocal microscopy.

References:

Figure S1 (a)FE-SEM and (b) HR-TEM images of the carbon xerogel.
Figure S2 FTIR spectra of (a) the supernatant solution of MCNP–3 and (b) the residues of MCNP–1, MCNP–2 and MCNP–3, after the complete removal of their corresponding supernatants.
Figure S3 XRD pattern of MCNP–3.
Figure S4 Maldi TOF-Mass spectrum of MCNP-3
Figure S5 The UV-visible absorption spectra of (a) as prepared MCNP–3 at pH 1 and (b) MCNP–3 at different pH ranging from 1 to 14. PL-spectra of (c) CNP producing by varying the concentration of nitric acid, (d) MCNP–3 against with the time of combustion starting from 12 hours to ending at 7 days, (e) MCNP–3 (generating after 72 hours of the reaction) at different pH, indicating the maximum fluorescence intensity at pH 5, (f) MCNP–3 exciting at different excitation wavelengths. (g) Digital camera images of different as synthesized supernatant solutions obtaining by variation of the concentration of nitric acid and their corresponding photograph under the exposure of UV-light demonstrating that they emit bright green fluorescent light under the excitation of UV-light. The cubates labelling as (i) and (ii) were the dilute solution of MCNP–1 and MNCP–2 respectively under the irradiation of UV-light.
Figure S6 (a) HR-TEM and (b) the corresponding SAED pattern of MCNP–2.
Figure S7 HR-TEM image of MCNP–4.
Figure S8 (a) low and (b) high resolution TEM images of MCNP–1.
Figure S9 Zeta potential of (a) MCNP–3 as a function of pH and (b) MCNP–1 to MCNP–4 at pH 1, 5 and 9, reflecting the fact that owing to the highest zeta potential value of MCNP–3 among all of its corresponding carbon nano materials, it was most disseminated in its aqueous solution thorough electrostatic repulsion and hence formed the smallest discrete particles giving the maximum fluorescence intensity.