Activation of aerial oxygen to superoxide radical by carbon nanotubes in indoor spider web trapped aerosol

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Domestic spider webs trap partly burnt combustion contaminants in indoor floating aerosols. These are shown to possess defective carbon nanotube particulates containing very stable carbon centred free radical. When inhaled the carbon radical readily activates aerial oxygen to superoxide radical, thus demonstrating the possibility of spontaneous intracellular generation of reactive oxygen species in intact cells.

Keywords: Aerosol, multiwalled carbon nanotube, reactive oxygen species, spin frustrated carbon nanotube, superoxide radical.

A major part of the personal exposure occurs in enclosed environment as people spend approximately 90% of their time indoors. Indoor air pollution is assessed from the available chemicals and dust settled in a confined place[1]. This includes combustion sources such as solid fuels, biomass, oil, gas, kerosene, coal needed for energy; tobacco, candle, faulty electric gadgets, incense and products from diverse human activities. Countries in the southern hemisphere extensively use room ventilators (fans) to combat heat and sultry weather. Within weeks, the blades of these ventilators turn black with the deposition of combustion particulates. The ingredients present in domestic airborne aerosols play a significant role in producing toxicological effects. Being sufficiently small and insoluble, these get adequate time to penetrate the deepest areas of the lungs triggering asthma attacks and aggravate suffering. Respirable particulate matter cause lung inflammation by inflammasome activation known to be triggered by reactive oxygen species (ROS)[2]. Nano-sized carbon black aggravates neurodegenerative diseases[3]. Hypotheses to identify the responsible particles have focused on shape and size, content like silica, asbestos or heavy metals, bio-aerosols and black carbon related particles. Spider webs were used to trace heavy metals associated with motor vehicle emissions[4,5].

We used domestic indoor spider webs (Figure 1) to capture airborne combustion products confined in the indoor space. These spider webs varying from one day to two weeks old were collected from inside of houses within the residential area in the campus of Indian Institute of Technology Kanpur (IITK; 80.20′E and 26.26′N) during June–December 2007. The contaminated spider web with airborne particulates was subjected to analysis in raw form and also after chemical treatments to clean the particulates and acid soluble metal ion contaminants from the web silk. With the probes SEM (scanning electron microscopy) and EDAX were recorded with FEI Quanta 200 Hv and Tecnai 20 G2 200 kV. STWIN was used for TEM (transmission electron microscopy) analysis and XRD (X-ray powder diffraction) and the AFM (atomic force microscopy) were carried out with Pico scan Model (Molecular imaging, US) in air under ambient conditions at room temperature. Silicon nitride tip (micromesh) was used and the size of the cantilever tip (radius of curvature) was less than 10 nm. The spring (force) constant of cantilever was 1 N/m. The images were taken in noncontact mode and the EPR (electron paramagnetic resonance) measurements were recorded at room temperature using a Bruker EMX spectrometer with microwave frequency 9.8 GHz, modulation amplitude 10 G, modulation frequency 100 kHz and microwave power 0.21 mW. Electronic spectra were recorded using a PE-Lamda-35 UV–Vis spectrometer and Raman spectra using WITEC model Raman spectrometer.

Figure 1 shows the domestic spider with its web and Figure 2 *a* shows its EDAX analysis with detectable presence of C, N, O, Mg, Al, Si, Cl, Na, K, Ca, Pb and Fe. The collected web materials were washed with alcohol to remove greasy matter, air dried and the residue was treated with 6 M HCl at 108°C for 16 h to hydrolyse the silk. The soluble part was centrifuged off and the remaining residue was washed with water to ensure leaching out

Figure 1. Domestic Indian spider with its web indoors.

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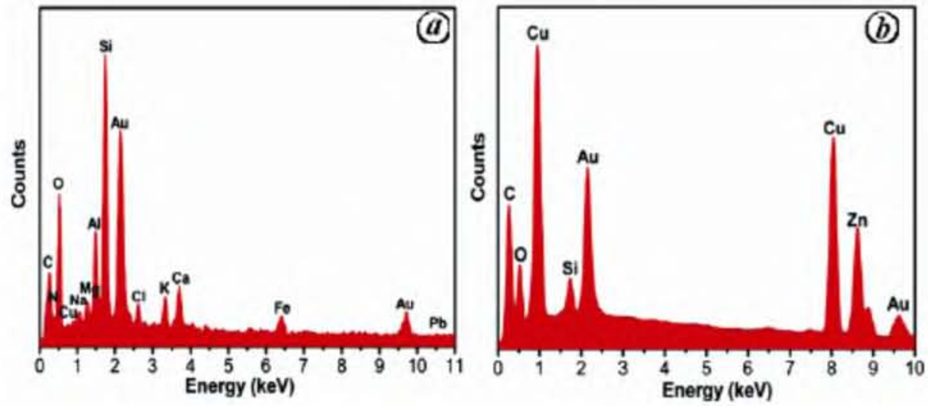

Figure 2. *a*, EDAX identifies the elements present in the raw spider web. *b*, after treatment.

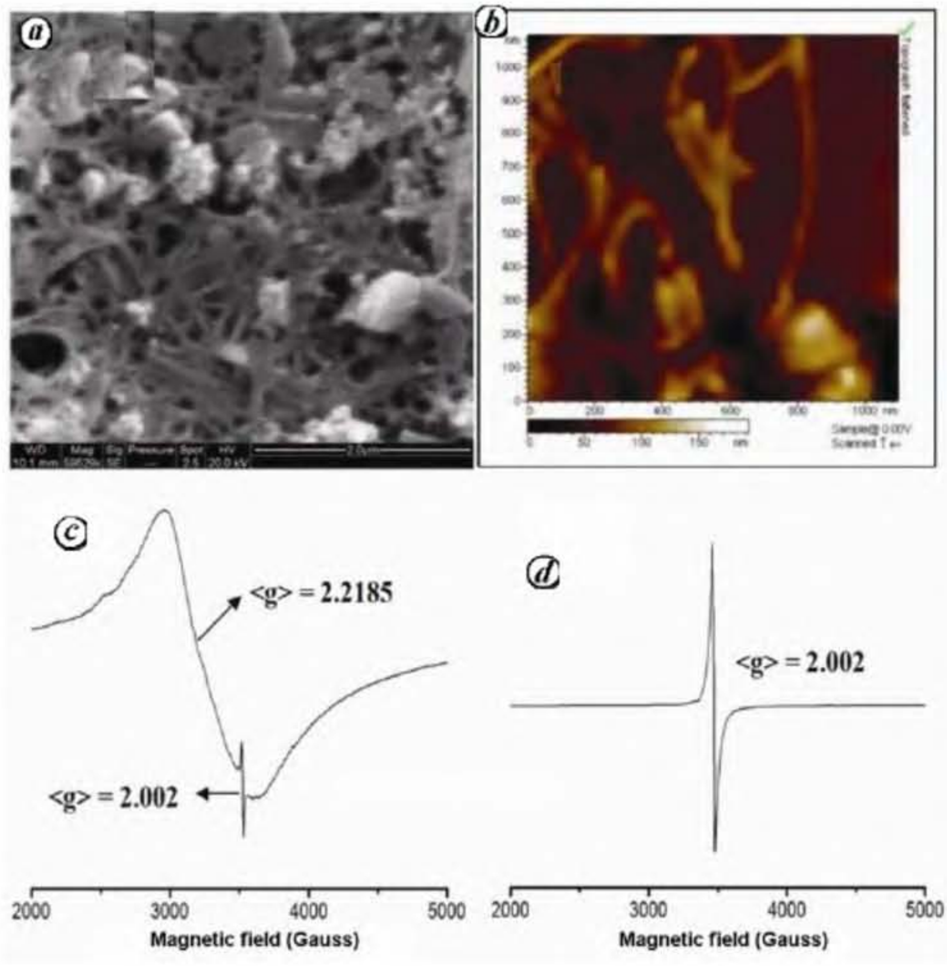

Figure 3. *a*, Scanning electron micrograph of insoluble black residue trapped in spider web freed from the protein and other acid soluble organic components showing the presence of carbon nanotubes and silica. *b*, AFM images of the same insoluble residue showing the presence of carbon nanotube (CNT). *c*, EPR of the raw spider web showing the presence of two EPR active species, the broad EPR is due to ferric oxide. *d*, Insoluble black residue conserving the EPR active CNT.

of traces of organic components and any residual soluble metal contaminants. The insoluble black residue was centrifuged, washed several times with water and air dried. The EDAX of the cleaned particulates showed the presence of only carbon, silicon and oxygen as shown in Figure 2 *b*.

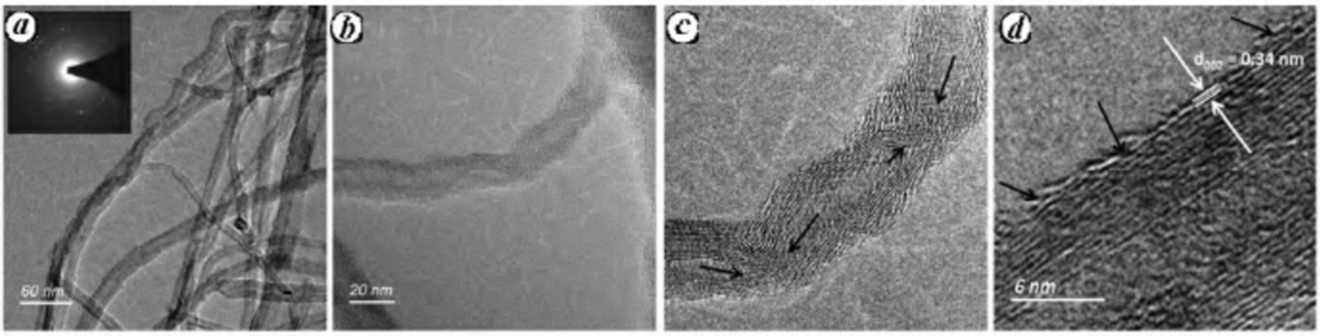

Figure 4. *a*, TEM image of the insoluble black particulates from spider web showing MWCNT with junctions and kinks (inset XRD). *b*, HRTEM image. *c*, Displaying defects in channels with blockage (black arrows). *d*, Broken outer edge marked with black arrows, also showing interlayer spacing in the MWCNT of 0.34 nm, corresponding to the (002) plane of graphite carbon.

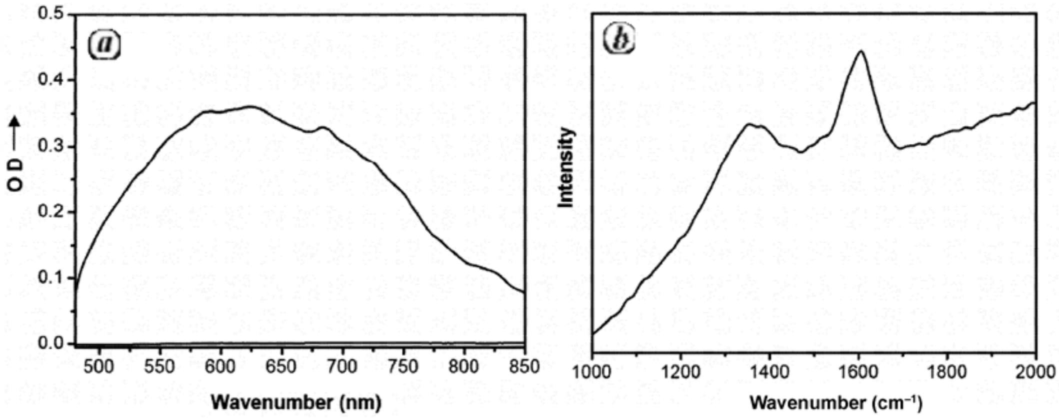

Figure 5. *a*, Nitro blue tetrazolium (NBT) test showing the formation of blue mono formazan dye from the interaction of insoluble black residue with air, a blank test in absence of this residue showed no development of blue colour. *b*, Raman spectrum of insoluble black residue showed the presence of sp^3 hybridized carbon along with sp^2.

The black residue showed the presence of only carbon, oxygen and silicon by EDAX (not shown) with the elimination of other trace elements found in untreated EDAX (Figure 2 *a*). Corresponding SEM image revealed the presence of carbon nanotubes (Figure 3 *a*). Its AFM (Figure 3 *b*) supports the presence of carbon nanotubes accompanied by some silica lumps.

The TEM image of the purified residue showed the presence of multiwall carbon nanotube (MWCNT) with relaxed diameter 20–50 nm range with several turns and junctions (Figure 4 *a*). Further, its high resolution transmission electron microscopy (HRTEM) image showed uneven obstruction present in the channels (Figure 4 *b* and *c*). Figure 4 *d* showed at the outer surface of the MWCNT several defects (pointed out by black arrows) and the interlayer spacing in the MWCNT, ~3.4 Å has been marked which corresponds to the 002 distance of graphitic carbon[6]. Defects like turns and kinks may cause bonding frustration in graphene's carbon leaving trapped carbon centred radicals in defective MWCNT[7]. Such radical species are readily identified by EPR spectroscopy. The raw web particles showed room temperature EPR spectrum (Figure 3 *c*) comprising one broad and one narrow signal. On washing with dilute hydrochloric acid in the cold, the raw web loses most of the metals present, particularly iron. This gently acid-washed residue or the final residue (after hydrochloric acid treatment under pressure to hydrolyse and to remove the silk proteins) showed identical EPR spectrum retaining the narrow resonance with $\langle g \rangle = 2.002$ (Figure 3 *d*). This evidently supports the fact that we are dealing with a stable EPR active species. It is interesting that this EPR active species is retained in the raw spider web even after harsh acid purification treatments suggesting its stability under the graphene matrix. This can happen when carbon nanotubes (CNTs) are associated with defects and turns and kinks[7]. These CNTs may be termed as spin frustrated carbon nanotube (SFCNT).

Interestingly, these SFCNT show spectacular properties on exposure to aerial oxygen. SFCNT slowly activate oxygen present in air to produce superoxide radical, which is a potent reactive oxygen species (ROS). We confirm this reaction by nitro blue tetrazolium test[8] (Figure 5 *a*). Raman spectrum of these SFCNT (Figure 5 *b*) showed the presence of sp^3 carbon along with sp^2, suggesting the presence of defects in graphene structure. The inhalation of these nanosized SFCNT particles which are floating inside the room may be effortless in breathing.

Recently, graphitic carbon was shown to catalyse aerial oxidation[9] and this may correlate the role of nanosized carbon black affecting neurotransmitter levels and pro-inflammatory expressions[3]. In case of graphitic carbon, its nonavailability in nanosize form indoors prevents its inhalation. This is not valid for SFCNT as they readily float in the air, according to our present observation. This study showed that indoor spider webs within days collect matter by trapping considerable amount of floating particulates inside a room which are potent materials for uptake by effortless human breathing. The catalytic action of SFCNT in generating superoxide radical strongly suggests that these may trigger direct inflammasome type activation[2]. Thus, these abiotic SFCNT may imitate impulsive pseudo phagocytosis.

Degradation of SFCNT is difficult and its size may not allow ready precipitation causing its gradual accumulation in indoor aerosols which on exceeding a threshold may lead to catastrophic consequences.

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ACKNOWLEDGEMENTS. This work is supported by a DST, New Delhi project grant from the Nano Science and Technology Initiative Scheme to S.S. S.K.S. thanks IITK for the Senior Teaching Assistantship and S.T. thanks UP State Council of Science and Technology, Lucknow for the JRF.

Received 12 Mach 2009; revised accepted 8 September 2009