

Water soluble nanocarbon materials: a panacea for all?

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The low solubility of nanocarbon materials (fullerenes, CNTs and graphene) has restricted their widespread commercial use. Functionalization, however, helps overcome this major limitation. Much work has been documented recently on water soluble fullerenes (FWS) in treating cancer (e.g. breast cancer), tumours, arthritis, Parkinson's, influenzas and HIV-AIDS. Even an aqueous nasal spray to combat Alzheimer's has been advocated. A recent US patent shows that FWS, based on its anti-oxidant activity, is a powerful Radical Oxygen Scavenger. FWS have been used in X-ray imaging, as MRI Contrast Agents (MRI-CA), to make solar cells and could help produce nanodevices and biosensors. This review examines this emerging field.

Keywords: Nanobiotechnology, nanomaterials, theranostics, targeted drug delivery.

NANOCARBON materials (NCMs) include fullerenes, carbon nanotubes and graphene, which have been recognized by the Nobel Prizes in 1996 and 2010. However, limited solubility restricts their widespread use. Functionalization of NCMs makes them soluble and thus in recent years the emphasis has shifted to water-soluble derivatives, which are relatively safe¹. Soluble NCMs have been shown to possess activity against cancer (e.g., breast cancer), tumours, arthritis, HIV-AIDS, influenzas, etc. There are even reports of their possible use in aqueous nasal sprays (containing up to 0.0002 mg of water soluble fullerene per 100 ml water) for treatment of Alzheimer's disease. The poster boy fullerene derivative, C₃-tris malonic acid possesses activity against Parkinson's disease in mice. A recent patent documents their use as antioxidants, as these are Radical Oxygen Scavengers (ROS), which are expected to serve as good theranostic platforms.

Is it true that water soluble NCMs are a panacea for all diseases? Though recent work seems to suggest this to be true, such claims need to be carefully evaluated. Water solubility would allow intravenous injections to patients, permitting targeted delivery to the cancer/tumour sites rather than spreading it all over the body. These are thus good candidates for personalized medicines, but toxicity issues should not be overlooked. We must remember that public perception and expectation could differ considera-

bly from experts' viewpoints. Soluble NCMs have been used in making solar cells, as MRI contrasting agents and in X-ray imaging. Nanodevices hold great promise, but just like the nanocarbon materials themselves, these still remain restricted to research laboratories, occasionally catching media attention. However, no one can deny that the time is ripe for them to reach the market soon.

Synthesis and characterization of nanocarbon derivatives

Buckminsterfullerene was discovered in 1985 by W. Kroto and others who received the Nobel Prize 11 years later. Fullerene C₆₀ is a spherical molecule containing six-membered aromatic rings and five-membered radialene rings. It is a good electron acceptor and has been studied using cyclic voltammetry. Limited solubility has, however, hampered its industrial use. The molecule has a diameter of 0.7 nm. Since it does not contain any hydrogen atom, it shows no signal in ¹H-NMR, except for the proton present on the substituent. It shows only one signal in its ¹³C-NMR spectrum at 145 ppm, showing that all 60 carbon atoms are equivalent. Functionalization by different methods of derivatization makes it soluble and derivatized fullerenes show no cytotoxicity. However, on derivatization the ¹³C-NMR spectrum is characterized by the presence of up to 60 peaks or 16 peaks between 137 and 143 ppm, depending on the symmetry of the product. Cost of production of fullerene in the past has also been a limiting factor. In recent years, an Arizona based company is selling C₆₀ at \$20 per gram, when a minimum of 100 g of this fullerene was purchased. The cost is expected to drop dramatically, when fullerene-based products reach the market and are found to be useful. FT-IR spectra and thermogravimetric analysis (TGA) are also used for its characterization. The FT-IR of C₆₀ shows peaks just like alkenes or aromatic rings. Two methods of preparing derivatives are the Prato reaction (reaction with nitrenes) and the Bingel reaction (reaction with carbenes), and they are commonly used for this purpose.

UV-Vis spectrum of C₆₀ shows bands at 213, 230, 257, 329 and 406 nm. After the Prato and Bingel reaction, a weak peak due to a singlet-singlet forbidden transition is seen at 430 nm. Modern mass spectrometric methods are extremely useful for characterizing fullerene adducts and precise molecular weights up to the fourth decimal place

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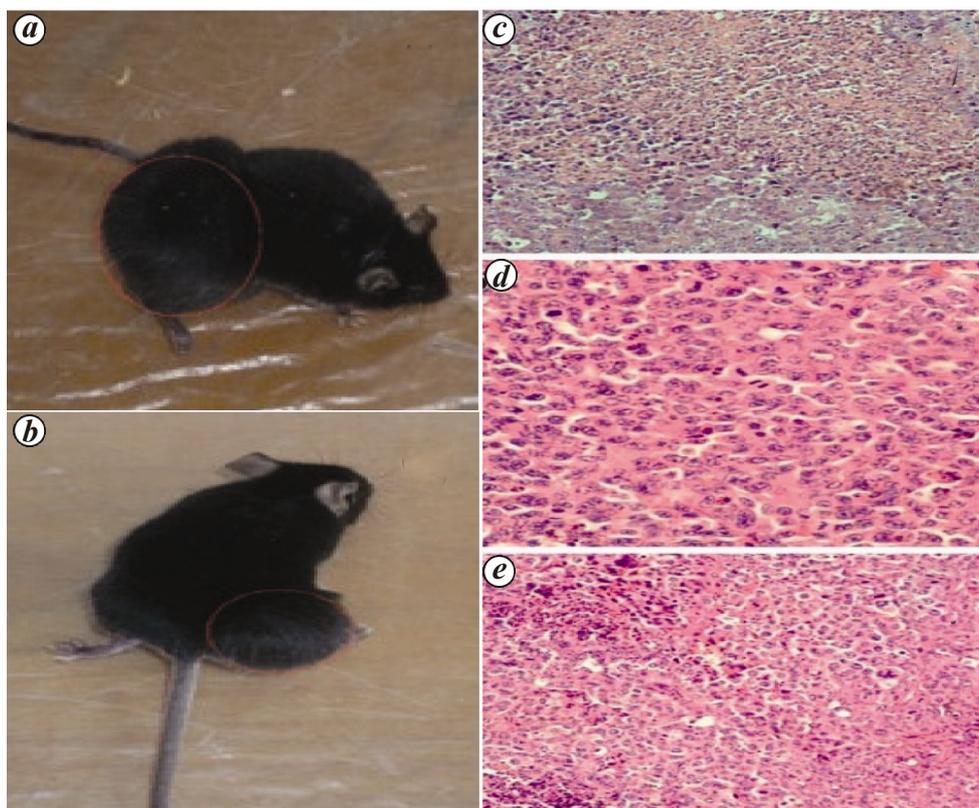


Figure 1. *a*, Visual comparison of tumour size of animals of group 2 (inhibitive effect) and *b*, group 1 (protective effect). Histological sections of Lewis lung carcinoma in male mice: *c*, control; *d*, Protective effects – mitosis and apoptosis; *e*, Inhibitive effect – areas of necrosis of tumour cells³.

can be obtained. The unreacted fullerene can be eluted from silica gel columns on elution with toluene followed by elution with 3 : 1 methylene dichloride : toluene. However, the nanoadducts of fullerene can be precipitated from toluene/ortho-dichlorobenzene using petroleum ether or acetone. Both the Bingel and the Prato reactions have 80–90% success rate.

Common functionalization methods include heating with concentrated HNO_3 and H_2SO_4 in a temperature range of 85–115°C. This could partially damage the nanostructure. The carboxylic groups, thus generated make the material more water soluble and serve as points where further reaction (SOCl_2), and attachment of other molecules like peptides and drugs can be carried out. However, the harsh acidic conditions used are better avoided. Instead, it would be prudent to use the Prato or the Bingel reaction, which could then be followed by the functionalization of the substituents thus added to the nanocarbon. This would permit such functionalization to be carried out with less damage to the NCMs.

An exceptionally quick method for preparing water soluble polyhydroxylated fullerenes involves reaction in 3 minutes with aqueous sodium hydroxide and a small amount of tert-butyl ammonium hydroxide as a catalyst. An average of 26 hydroxyl groups are thus attached, leading to greater water solubility.

Derivatives of other nanocarbon materials, viz. CNTs and graphene are characterized using FT-IR and UV-vis, TGA, TEM, SEM, cyclic voltammetry and Raman spectroscopy.

Water soluble fullerenes for medical applications

It is known that fullerene shows little toxicity in its functionalized form. This is despite the possible cytotoxicity arising from the efficient generation of singlet oxygen. Fullerenes are powerful antioxidants due to their ROS activity² and they can serve as multifunctional theranostic (therapy + diagnostics) platforms ‘demonstrating the beauty and power of nanotechnology’. It is necessary to emphasize that fullerene is homogeneous as it represents a single molecular structure with precise molecular weight and 3D-structure, which is very important for the pharmaceutical industry. Unlike fullerene, ‘the length and chirality of CNTs may vary’ and graphene sheets may be ‘in different size and termination’.

The use of water-soluble C_{60} fullerene in anticancer therapy has been described³. Being water soluble it could be used for intravenous administration with the active agent being made available only at the desired site and not circulating throughout the body⁴. These studies

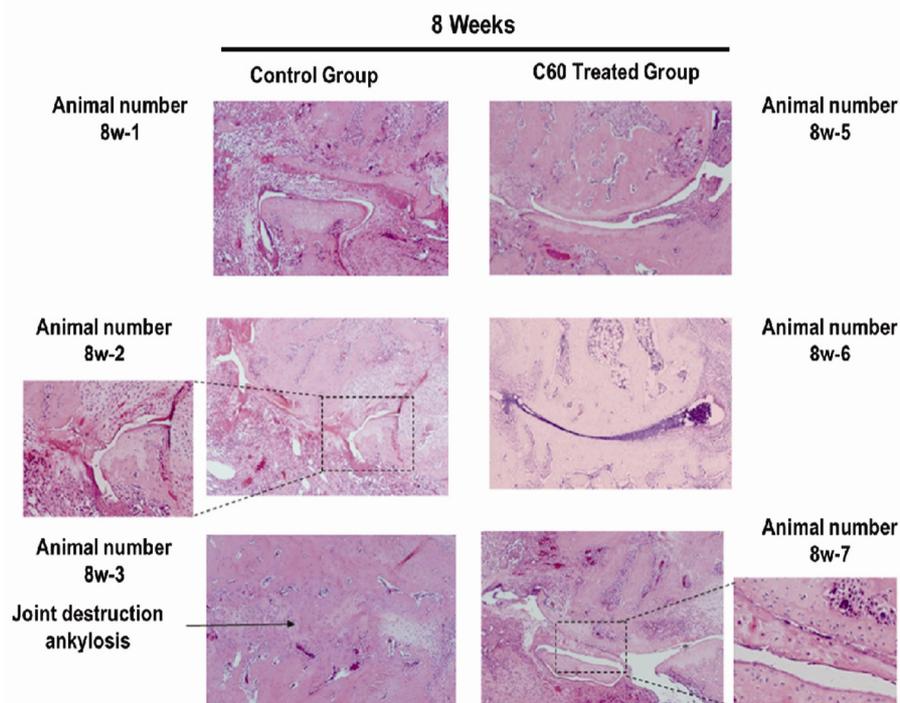


Figure 2. Representative images of the joint damage at each time point in arthritis model rats. At the eight-week time point, all ankle joints that had been treated with PBS showed severe joint destruction and bony ankylosis. In contrast, inhibitory effects were observed following administration of C_{60} in the C_{60} -treated group⁸.

transplanted a tumour in mice and studied anticancer activity in mice. Histological sections of mice lung carcinoma in male mice, 34 days after transplantation were studied (Figure 1). They divided the study into two parts: when the C_{60} derivative was injected into the animals before tumour transplantation, it was called ‘the protective effect’ and when it was injected after the transplantation of the tumour it was called ‘the inhibitive effect’. It is noted that in ‘the protective effect’, the C_{60} derivative activated mitosis and apoptosis of tumour cells. As against this, in ‘the inhibitive effect’, C_{60} activated necrosis of tumour cells. When treated with 5 mg/kg in lung carcinoma in male mice C57BL:J in ‘the protective effect’, 35.3% of tumour growth inhibition was observed. In ‘the inhibitory effect’, 25.1% tumour growth and extension of life by 21.8% were observed. In the metastasis ingestion effect, 96% for ‘protective’ and 48% for ‘inhibitive’ effect of C_{60} were observed, which ‘is promising for clinical oncology’.

A US patent in 2016 established the antioxidant property of water soluble fullerenes⁵. In addition to FT-IR, UV-Vis, Zeta potential, MTT assay (cytotoxicity assay), TEM, AFM, SEM were recorded. Electron paramagnetic resonance (EPR) measurements are particularly noteworthy for establishing the radical quenching property. The superoxide generated was quenched completely by C_{60} abolishing the EPR spectrum. The MTT assay established that carbon nanomaterials are not toxic. Chemotherapy of breast cancer depends on whether it is carried out before

surgery (neo-adjuvant) or after surgery (adjuvant) or whether the cancer is in an advanced stage. Currently used drugs include, Doxorubicin (Adriamycin), Taxotere and Carboplatin. However, all these drugs have severe side effects. Doxorubicin shows severe cardiotoxicity leading to permanent heart damage (cardiomyopathy). However, soluble fullerene C_{60} modulates such side effects of Doxorubicin. Gadolinium metallo-fullerenol nanomaterial was found as a nontoxic breast cancer stem cell specific inhibitor, showing higher anticancer activity than Cis-platin. Intracellular release of doxorubicin is tracked by fluorescence quenching by C_{60} . Photodynamic therapy (PDT) has also been considered, but C_{60} has a low efficiency at the commonly used wavelength of 532 nm. Though it absorbs well in the UV range, it does so poorly at the red end of the spectrum.

The poster boy of fullerene adducts, the C_3 tris-malonic ester C_{60} (at 14–15 μM) completely eliminated all superoxide radicals generated *in situ* and this one derivative is thus pushing the field ‘closer to the market’. This compound when administered to non-human primates with Parkinson’s disease improved their motor function⁶. Beneficial effects of polyhydroxy fullerenes (fullerols or fullerlenols) on growth and lifespan in diverse biological models have been discussed⁷. Fullerene water solutions (FWS) containing 0.002% mg C_{60} per 100 ml, 0.002 mg of FWS/100 ml of water have been approved as dietary supplement by the Ukrainian Ministry of Health. Patients may spray this water as nasal spray and drink certain

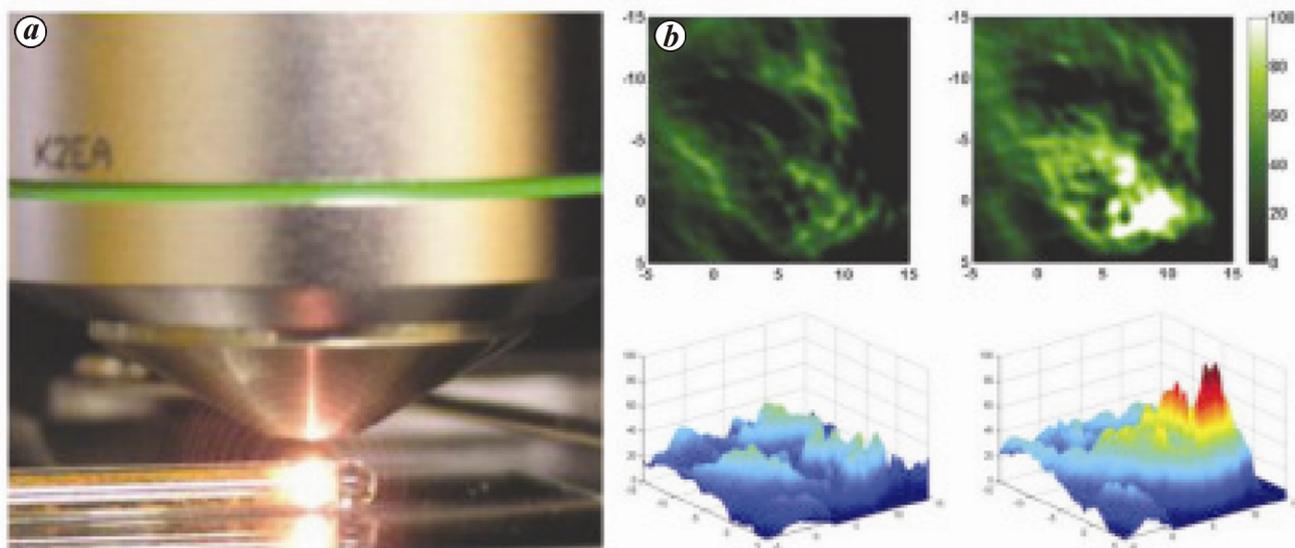


Figure 3. *a*, Visible light emission from a soluble fullerene irradiated with a 785-nm laser. *b*, Photoacoustic imaging and 2D signal mapping of tumours in living mice before (left) and after (right) intra-tumoural injection of soluble fullerene¹⁷.

amounts of this water to overcome the ill effects of Alzheimer's disease. Is 'Fullervir', an Elixir? Beneficial effects of water soluble C_{60} have been demonstrated in the rat model of arthritis as it arrests arthritis in ankle joints (Figure 2)⁸. Should these results be extended to humans? Can suffering, swelling, pain and inability to move joints be considerably reduced? 'Fullervir', the sodium salt of polyhydroxy fullerene poly-amino-caproic acid, manufactured by Intel, showed anti-retroviral properties in human cell cultures infected by human immunodeficiency virus (HIV-AIDS). This agent was able to protect the cell from the cytopathic action of HIV inhibition of HIV-1 protease by fullerene based inhibitor, demonstrated very recently⁹. Water soluble fullerene shows beneficial effects on growth and diverse biological models. Effects of NCMs on plants have been documented in *Arabidopsis thaliana*. Interestingly, seed germination is promoted and increased length of hypo-cotyledons have been observed. This augurs well for agriculture, as the seeds could now be sown at greater depth and stronger plants could emerge from the soil. The distribution of nano-substances in different parts of the plant body has also been documented. This must caution us about possible distribution of nano-substances in plant bodies, soils and the environment. Presence of fullerene-like structures in Chinese hibiscus (*Hibiscus rosa-sinensis*) with putative function for mechanical stability and adductive property have recently been established¹⁰.

SWCNTs and graphene

Preliminary studies by Romanian scientists have shown that attachment of doxorubicin onto NCMs could be used for targeted release of doxorubicin. Though they show TEM, SEM pictures, and have studied the release of the

anticancer drug, their results are at best indicative in nature. A recent study from the Amrita Hospital, Edapally (Kerala, India) conclusively proves that a drug like this could be attached to graphene and combined with photodynamic therapy (PDT)¹¹. Studies co-authored by Rao¹² lend great credibility to these results. The regression of tumours by this combined PDT, using graphene drug therapy is thus well established by this group.

Application to materials science

Efficient solar cells have been fabricated using water soluble fullerene. Similarly gadolinium C_{60} adducts have been found useful as MRI contrast agents¹³. FWS containing multiple carboxylic groups show excellent magnetic properties and have also been used for nanolithography. Gadolinium chelates are commonly used for MRI scans in hospitals, but the toxic gadolinium can be released from the chelate inside the patient's body. Fullerene-gadolinium adducts do not suffer from any such limitation. It is thus expected that very soon this Gd- C_{60} will replace Gd-chelates. Hydrogen absorption ability of the FWS is very superior to other alternatives and may, in future, be used to fuel automobiles. A car of nanodimensions with fullerene as wheels has also been conceived! Indeed, using infrared spectroscopy, fullerenes/fullerene adducts have been detected 'in the circumstellar envelopes around low-mass evolved stars' (so-called planetary nebulae)^{14,15}.

Fullerene based sensors for theranostics

Soluble derivatives of fullerene add a new dimension to the construction of highly sensitive biosensors¹⁶. These

have been used to detect glucose levels in blood serum, urea levels in urine, haemoglobin, immunoglobulin, glutathione in samples for pathological purposes, to identify doping abuse, to analyse pharmaceutical preparations, and even to detect cancer and tumour cells at an early stage. Thus theranostic applications of water soluble fullerenes in photo-acoustic imaging have been described (Figure 3)¹⁷. Questions are raised as to how far we have progressed with nanodevices. Yes, they make good headlines in the media and are good materials for research proposals, but what is the reality? How far are we away from marketable, useful nano-devices? It is to be hoped that both in medical and materials science applications of water soluble fullerenes, there will be much progress in coming years.

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