

# From Coal to Nanocarbon to Cross Blood Brain Barrier to treat Alzheimer, Parkinson and Brain Tumour

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**A**LS, the disease that Stephen Hawking suffered from, is a motor nerve controlled by neurons in the brain or Olympic gold medallist Mohammed Ali surrendered to a fatal disease named Parkinson. Similar to these, there are other brain diseases like Alzheimer, Schizophrenia to Neuralgia and brain tumour affecting people equally poor or rich, common or celebrities. All these diseases cannot be cured till today as we cannot send a drug inside the brain because of the “blood brain barrier’ (BBB). This natural barrier will not allow any foreign body even a drug to cross it to reach the brain. Is it possible to open the skull everyday and put a drug inside for a cure? No, that is not the remedy, so we developed an alternate strategy to create a box like hollow cage which can cross BBB, hiding any drug inside and will deliver in the brain and after delivery will be excreted from the body. We use a very cheap source to design this box from low grade coal abundant in our country costing 5 rupees a kilo!

The perivascular cells in the brain play an important role of selective permeable space between the blood circulatory system and central nervous system which is commonly known as the BBB. It is composed of endothelial cells, pericytes and astrocytes and it protects the functionality of the brain and central nervous system (CNS). Pericyte cells create the BBB with tight junctions to protect vesicle trafficking through the endothelial cells and inhibit the effects of CNS immune cells and pericytes as contractile cells also contribute to controlling the flow within blood vessels as well

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\* Mr Bholanath Pakhira, Ph.D. Scholar from Indian Institute of Engineering Science and Technology, West Bengal, is pursuing his research on “Graphene and Nano Carbons from Coal and their Use as Cargo for Drug Delivery including Passage through Blood Brain Barrier.” His popular science story entitled “From Coal to Nanocarbon to Cross Blood Brain Barrier to Treat Alzheimer, Parkinson and Brain Tumour” has been selected for AWSAR Award.



*Figure 1: Schematic role of BBB*

as between blood vessels and the brain. So this BBB due to its neuroprotective role not only blocks the unwanted stuff to get in to the brain but also any drug molecule.

Lipid (fat) soluble (hydrophobic) molecules may penetrate into the brain. Moreover, drug molecules need to be carried by aqueous blood plasma and these should be hydrophilic. So the box environment has to be created which should possess amphiphilic property and this can flow through blood plasma and when it reaches the lipid gate can enter there as well. The width of the extra-cellular space in BBB is 38–64 nm so the box should fit in.

Therefore, the essential need for such a box:

Synthesis of non- toxic box having maximum 64 nm diameter size

It should be amphiphilic (water and lipid soluble)

It should be non- toxic

It should be self fluorescent (beyond auto fluorescence) to monitor its location

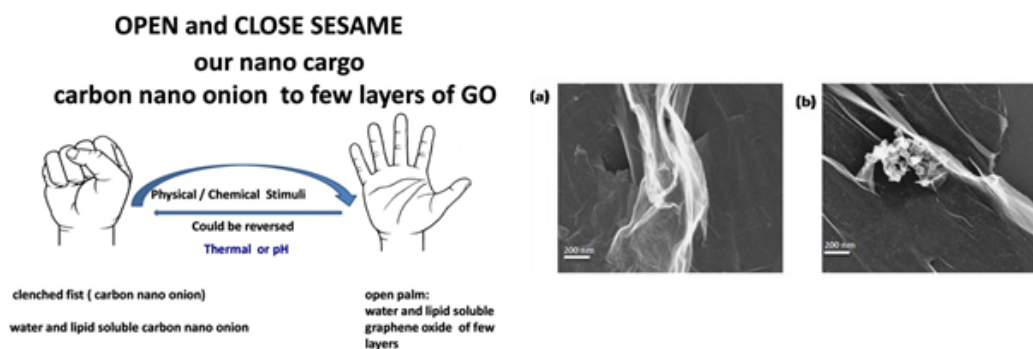
It should work like a box which can open and close to carry inside anything smaller to its size and can load and de-load under proper stimuli

It should be readily excreted out after its job is done.

We at Nanoscience and Synthetic Leaf Laboratory at Downing Hall, IEST, Shibpur, West Bengal, under the mentorship of Prof. Sabyasachi Sarkar developed a new type of carrier (water soluble carbon nano onions, wsCNO) from low grade coal, yes you read it right, low grade coal which is not even used by steel industry for its low caloric value which produces our desired nano carbon cargo (box) to cross BBB. We can now deliver any medicine inside the brain and can monitor it.

### How was this study initiated?

In 2005, water soluble carbon nanotube was first reported by us in the form of Kaajal<sup>[a]</sup> and it was predicted that such system can be utilized in biomedical applications. We were able to make different types of water soluble carbon nano particles like onion (wsCNO) with photo



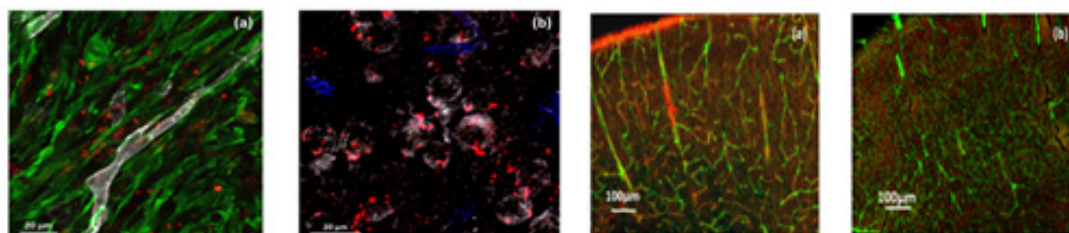
**Figure 2:** Smart Molecules changing shapes: SEM images of GO prepared from coal, (a) freshly prepared, (b) after few hours changing to sphere, ; interchanged by pH variation

luminescent properties from cheap sources like wood, wool or plant waste by carbonizing these followed by oxidative treatment. We were successful to use these wsCNO for in vivo imaging of the entire lifecycle of the fruit fly (*Drosophila melanogaster*)<sup>[b]</sup> also we showed that such nano carbon can feed micro nutrients as well as adsorbed water to young saplings even in arid zones “ like spoon feeding “ as cited in our work by Chemistry World.

Finally, we extracted preformed graphene oxide (GO) from low grade coal with size distribution in the range of 40 to very large in nm. Interestingly these corrugated sheet type GO under aging or any stimuli changed to spherical shaped wsCNO. We optimized the size of such GO under nitric acid nicking to get their shape less than 35 nm in size.

Such wsCNO exhibits pH dependent, open and close form or can be thermally controlled.

Just to understand the utility of our wsCNO as box (Trojan horse) to cross BBB we performed animal study; 6-8 month old transgenic mice were induced with glioblastoma multiforme, a type of brain tumour, and CADASIL, a genetic disorder that contributes to vascular dementia in humans. The experiment involved injecting the wsCNO in the tail of the mice and imaging their brain. We have observed the passage of wsCNO to the tumour and also to the neuronal sites. A movie file



**Figure 3:** Left side, from brain slice : wsCNO reaching a) tumour (green fluorescent protein tagged, red our wsCNO, and white level neuron tagged,) and b) neuron sites in brain (blue Dapi blood channel); Right side, a) after an hr of wsCNO (red fluorescence) shows presence of wsCNO, b) mouse sacrificed after 3 days to show the clearance of wsCNO

showing live image of wsCNO movement has also been recorded (not shown for space restriction)

Thus we are delighted to establish that wsCNO cross through the BBB and enter the brain near neuron without causing any perfusion. This raises immense possibilities for drug delivery to the brain. These wsCNO do not accumulate in the brain but it is excreted from the body as analysed through the fluorescence study for wsCNO in the excreta for three days.

Now we have utilized the open form wsCNO to encapsulate desired drug molecules and its release. Some representative drugs were encapsulated inside the wsCNO in this study. Using electronic spectra, Drug@wsCNO composites were established and the release was quantified and almost 47% TMZ (Temozolomide) encapsulation i.e. in 10 mg composite 4.7 mg TMZ molecule could be found. In the case of Donepezil, an Alzheimer's drug it is 42%. These composites of wsCNO are soluble in PBS buffer and the retention and release have been found to be pH dependent. So we have successfully developed a cargo can be loaded with any desired drug or composite drugs and that can be injected through the blood vein and its movement can be monitored and near the affected cell sites the drugs may be deloaded under need- based stimuli.<sup>[c,d]</sup>

Previous work of our lab related to this work:

- a) Dubey P. et. al., Synthesis and characterization of water soluble carbon nanotubes from mustard soot, *Pramana J. Phys.*, (2005), **65**, 681-697. Indian Scientist make CNT for Kajor published in **Economic Times, 6th December, (2005)**.
- b) Ghosh M. et. al., Carbon nano-onions for imaging the life cycle of Drosophila Melanogaster, *Small*, (2011), **7**, 3170-3177. Glow imaging for living published in **Nature India, 8th November, (2011)**. Part of this work has been published as
- c) Pakhira, B. et al. Carbon nano onions cross blood brain barrier. *RSC Adv.*, (2016), **06**, 29779-29782, and highlighted in **Nature Indiaas Trojan horse to transport drugs to brain, March (2016)**.
- d) Pakhira, B. et al., Extraction of Preformed Graphene Oxide from Coal: Its Clenched Fist Form Entrapping Large Molecules, *RSC. Adv.*(2015), **05**, 89076-89082.