# Water Soluble Carbon Dots for Target Cancer Drug Delivery & Bio-Imaging

Af. Allam*, Ar. Allam* and S. Sarkar**

* Cromoz Inc, 2 Davis Drive, Research Triangle Park, 27709, N.C, aallam@cromoz.com

**Bengal Engineering Science University-Shibpur, Botanic Garden-711103, Howrah, India, protozyme@gmail.com

## ABSTRACT

Biologists for years have been thinking to visualize the movement and action of proteins and other molecules in and around cells and tissues. Water-soluble carbon nano-materials are introduced as a nontoxic, fluorescent reagent; enabling a living species to be imaged alive. This extends the possibility of tagging drug molecules so that they can be delivered to the proper site guided by image movement of the fluorescent tag. Our research has produced water soluble fluorescent multi-walled Carbon Dots ( C. Dots) and water soluble carbon nanotubes (wsCNTs) by functionalization of the surfaces and with no external passivation of surfaces with any organic wrappers. These carbon nano materials are produced from simple natural organic resources on pyrolysis and not from the commonly used laser ablation techniques on graphitic carbon. We have successfully conjugated these water-soluble functionalized C.Dots and CNTs with Paclitaxel and Gemcitabine. Initial *in vitro* studies on certain cancer cell lines at Johns Hopkins have shown no cytotoxicity.

***Keywords**:* Water Soluble C.Dots, WSCNTs, Bio-imaging, Target Drug Delivery, and Conjugation

## 1 INTRODUCTION

Real-time X-ray and magnetic resonance imaging are known methods used for biomedical diagnosis. By the oral administration of barium ions, X-ray imaging can be extended for use in soft tissue imaging. The other approach is MRI. The use of fluorescent probe is a new approach for imaging within living species. The fluorophore is normally used as GRP protein and its gene is transferred to transgenic animal for fluorescence study. Alternate to this, highly fluorescent quantum dots are covered or wrapped by hydrophilic polyols and intravenously introduced into the body of the laboratory animal for imaging. It is known that within a few hours the hydrophilic (the conventional polyethylene glycol (PEG) ) polyols is removed from the dot because of biological reaction, and the animal is subjected to the toxicity of the semiconducting quantum dot. [1-7] To overcome this, the oral ingestion of a fluorescent probe is now used for imaging a living species.

It was Professor Smalley who first predicted the 'wet side' of nano carbon materials for potential applications in the biological field. It is a real challenge to make/produce carbon allotropes soluble in water, as these hydrophobic materials are inert, they do not to respond to any chemical treatment. In addition, there is a definite added benefit or incentive to produce these materials using low cost materials/sources . Electric arching, laser ablation or even CVD techniques are not routine or commonly used for any wet chemistry laboratory. To overcome these challenges, Cromoz Inc. in association with the laboratory of Indian Institute of Technology, Kanpur, developed a technique to create nano carbon derivatives and to derivatize these by introducing hydrophilic groups to make these water soluble. Our continued research in this direction presents a lot of possibilities, especially in facilitating the spotting of malignant tissues inside a living animal, for transporting the drug of choice to that site, and finally to deliver the drug with precision to the cancer cells. The object is to execute this entire operation in a non-invasive manner by leveraging the fluorescing capability of these nano carbon materials.

Scientists first developed the use of green fluorescent protein for bio-imaging purpose. The drawback to the use of these proteins lies in their instability. As these are proteins, they can readily get denatured and decomposed. To overcome this, an alternative method is used to incorporate the GRP producing gene and create transgenic species for self fluorescence. However, such methods are very lengthy and time consuming and can not be used in humans [8]. As an alternative to this, highly fluorescent quantum dots were covered by hydrophilic polyols and intravenously introduced into the body of the laboratory animal for imaging. It is known that within few hours the hydrophilic polyols is removed from the dot because of biological reaction and the animal is exposed to the toxicity of the semiconducting quantum dot [6]. In *in-vivo* tests QDs containing cadmium or other metals with sulfide or selenide ions are shown to be toxic to the cells. Hence, there is a need for a benign nanomaterial which is not only water soluble but also has similar optical properties of GRP and quantum Dots. To meet this objective, Cromoz Inc. has developed a now patent approved method to create fluorescent water-soluble carbon dots (C. Dots). These C. Dots have been oxidized at the surface  to introduce hydrophilic groups to create water-solubility. In their nascent form they also exhibit fluorescent capabilities without the need to add any surface passivation. This now becomes a viable source to be used in applications beyond those of their cadmium-selenium (CdSe) counterparts.

## 2 MANUFACTURING PROCESS OF VALUE ADDITION PRODUCTS

Several applications of carbon nano materials have appeared in the bioengineering field. These applications include: biosensors, protein detection, DNA detection, chemical immobilization, and cancer drug delivery to target site. However, because all carbon materials are hydrophobic and water insoluble, (including organic solvent, though biology requires only water as its solvent to carry out biochemical reactions) the utility of  carbon nano materials  has some serious limitations to their biological application. The ubiquitous solvent in biology is water.  Accordingly, there have been attempts at preparing water-soluble CNT. One way to create  CNT  to be dispersed in water is to  wrap these by organic polyol molecules  so that  these go readily in water. In US patent application 2006/0003401 there is described a CNT that has been wrapped with a so called "self-assembly material". However, the CNT itself is not water-soluble and once un-wrapped the CNT is thrown out from its environment. And such wrapping molecules may unwrap rapidly under the interaction of the  bio fluid during their use.

In this context Cromoz made the discovery that CNTs prepared by a simple method like pyrolysing organic materials yielded surface defective CNTs. These defects readily allow such CNTs to get oxidized and randomly introduced high-density carboxylate groups on its surface. Such derivatives by virtue of several hydrophilic carboxylate groups made the carbon nanotube  water-soluble. These are multi layered CNT and so only the top walls ( exposed surface) gets heavily derivatized and the inner walls of these CNTs remain intact for further work. Such functionalization permanently wrapped the CNT by covalently linked hydrophilic groups and so water soluble CNTs are created and these do not have such unwrapping possibility once in use under bio fluid with time.  Using similar technique nano carbon dots were also prepared. C. Dots produced from hydrocarbon wax soot can be oxidized to produce side-walled incorporated carboxylic acid groups on the outer surface thus making the C. Dots water-soluble.  The passivation of the surface by the introduction of several carboxylic acid groups made these to have  quantum confinement effect and so these fluoresce.  As a result, these are produced in different nano sizes these fluoresce in multiple visible colors. These can be separated using nano filters or even can be directly be used for multi colored imaging.  It has further been discovered that these water-soluble nano carbon materials  can be conjugated with  a pharmaceutical composition. This may be used as carrier to drug and monitoring the movement of the fluorphore carrying the drug inside  and locating the place can be used as  a drug delivery system.

Cromoz Inc achieved the break-through to create  water-soluble multi-walled CNTs and  carbon dots starting from natural organic material  without the use of any toxic metal (Figure 1). This makes the CNTs and carbon dots very bio friendly, and viable for introduction into bio organisms. The functionalization of the wall of C. Dots provides the potential to attach a cancer drug (by conjugation) to be used in target drug delivery.

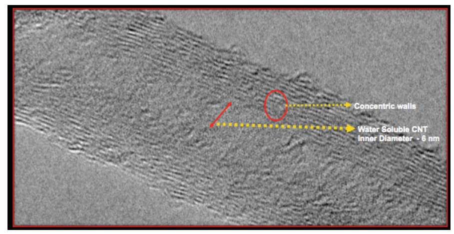


Figure 1: Multi-walled structure of water soluble Carbon Dots

## 3 WATER SOLUBLE CARBON DOTS

Collaborative research with the Department of Chemistry, at Indian Institute of Technology, Kanpur, helped Cromoz  to develop a technique to produce water-soluble carbon DOTs also known as Carbon Quantum Dots. This research work has been very significant, as these Carbon Dots fluoresce at different wavelength depending on the distribution of their size. The entire visible to near infra-red energy window is covered by these assorted water soluble C. Dots. This creates significant opportunity to market them as bio-imaging probes. The known fluorescent nano materials present in this field are not bio-friendly as they are found to be toxic in nature. Examples of those would include ZnS or CdSe and other similar semi-conducting materials. As Cromoz's Carbon Quantum Dots are produced from bio-friendly carbon source these  fluoresce natively without any wrappers.  These are very attractive as probes for the use in bio imaging that can image brain tumors or certain abnormal growth etc. and  thus may provide better diagnostic capabilities.

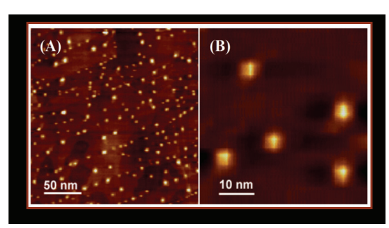


Figure 2: Atomic Force Microscopy images (AFM) of  water soluble Carbon Dots showing the size of C. Dots

Further studies with small bio organism like *E. coli* or even with mice show the Carbon Dots fluorescing in them (Figure 3).

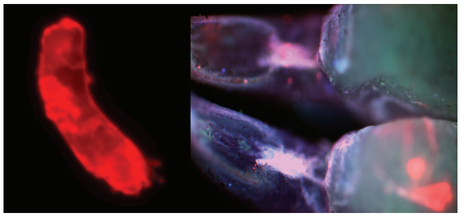

Figure 3: High Magnification Confocal Microscopic Image shows fluorescent *E. coli* and merged ( 385/488/561 nm ) image of mice finger tips upon oral ingestion of water soluble fluorescent Carbon Dots

## 4 OUR TARGET MARKET

### 4.1 CNT as Target Drug Delivery

Today, the most commonly used mechanism for delivering cancer drugs is through chemotherapy. This mode of delivery has significant side effects on the patient because the drug cannot differentiate between the benign and malignant cells. The side effects are often due to higher than required dosage level of the drug to compensate for the wastage before the drug reaches the target site. However, if a target delivery of drug localized to the malignant cells can be made, then the following benefits may be realized:

- Use only the required amount of drug needed. This reduces significantly side effects to the patient
- As only require amount of drug is used (say required 1 mg instead of 100 mg used today in chemotherapy) more patients can be treated
- Cost of chemotherapy can be brought down as only required amount (much less than what is currently used for chemotherapy) of drug can be used
- The benign cells are spared and so the side effects are minimized

Due to the above benefits, the pharmaceutical companies across the world are looking for and working on target drug delivery mechanism. We can provide these water soluble carbon quantum dots ( C.Dots) and water soluble CNTs to these pharmaceutical companies and other research laboratories with instructions on how to use these for conjugation with the potential drugs. Folate type groups can be linked with these C.Dots and CNTs where it is clear that the folate group acts as sensor to reach cancer cells. The movement of the drug conjugated C.Dots and CNTs can be tracked and imaged using fluorescence microscopy in small laboratory test animals. Cromoz can supply water soluble C.Dots and CNTs to the prospective companies and research institutions in solid form or even in dilute solution form for their further research depending on their specific needs.

### 4.2 CNT/CDots conjugation to Cancer Drug Service

We will provide customized conjugation service to pharmaceuticals companies in US, Europe and Asia. The companies will provide their potential cancer drugs and we will conjugate it using our CNT/CDots and send back to them the CNT/CDot conjugated drug. They can now use this for further research in their own labs.

### 4.3 CDoT for bio imaging and drug delivery

Our Carbon Dots have shown to absorb and emit at different wavelengths. This makes them very useful for bio imaging. As they are organically manufactured, they do not have any potential toxic affects like other Quantum Dots in the market today.

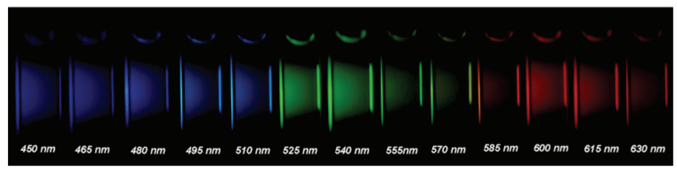


Figure 4: Emission spectra in increments of 20 nm wavelength.

Emission spectra (Figure 4) is in the visible-near IR region. Fluorescence profile under progressively 20 nm increment of excitation wavelengths from 360 nm of water-soluble carbon dots.

## 5 KEY STRENGTHS

- High solubility in water with good pore size.
- Can carry molecules and delivered to the site with a lag time dependent on the size of the CQD used and on the nature of hydrophilic/hydrophobic interaction.
- The bent and kinks present in these wsCQD directly make these points vulnerable for the subsequent use in opening up under the influence of biochemical reactions, which is relevant for its use in drug delivery. Hence, are highly useful as a material for drug delivery.
- Size and the shape of the materials can be controlled so that these can be used as material for selectively holding a specific drug for a specific period of time synchronizing its delivery time and approach to the site. These fluorescent probes are spontaneously excreted from the body.

## 6 COMPARISION

| GFP | METAL QUANTUM DOTS | CARBON QUANTUM DOTS (C.DOTS) |
|---|---|---|
| - Bright green fluorescence when exposed to blue light<br>- Excitation peak at wavelength 395 nm<br>- Emission at 590 nm (lower part of visible spectrum)<br>- Modified forms used as biosensors<br>- Fluoresce when Ca2+ ions present<br>- Short lived fluorescence | - Semiconductor<br>- Easily embedded in solid-state systems<br>- Manufactured from: Cadmium, Selenide, Lead and other metals | - Bio imaging<br>- Lasted for well over 20 hours<br>- Non toxic<br>- Solid form<br>- Administered orally, intravenously, or intramuscularly<br>- Absorb light in visible range 450 nm-630 nm<br>- Different size C. Dots absorb light at different ranges & emit a wide spectrum of color<br>- Useful for multi-color multiplexing<br>- Spherical |

## 6 NEXT STEPS

Our studies to date have been based on small microorganisms such as *E.coli*. We need to extend this study to larger test animals such as mouse and such possibility is proved by a tip experiment ( Fig.3).. This will enable us to provide better perspective on toxic effort of both our water soluble carbon nanotubes and Carbon Dots. This will require us either to setup our own animal test facility or outsource this to an external animal facility lab.

In addition, we would like to start conjugating our water-soluble carbon nano-materials with cancer drugs such as (a) Gemcitabine – used for pancreatic cancer treatment and (b) Taxol, which is commonly used anti-cancer drug. Introduction of folate groups also will help for selectively approach of these drug-loaded carriers to spot and reach cancerous cells. The use of vibrational energy may ultimately de-load the drug to the specific site.

## 7 PATENTS

Patents have been filed in US and India for both water-soluble carbon nanotubes and water-soluble carbon dots. These are important for the protection of our intellectual property.

(1) Title: Water Soluble Fluorescent Quantum Carbon Dots

a) Indian Application No.: 618/DEL/2011

b) US Filing Application No: Application No.: 12/719,791

(2) Title: Water Soluble Concentric Multi-Wall Carbon Nanotubes Us Filing Application No: 11/680,888

## 8 REFERENCES


[1] M. Felton, "Carbon in Quantum Dot-Land," American Chemical Society. 2006. http://www.clemson.edu/ces/lemt/Sun_InTheNews%20materials/quantumdotland.pdf

[2] Tsoi KM, Dai Q, Alman BA, Chan WC, "Are Quantum Dots Toxic? Exploring the discrepancy between cell culture and Animal Studies." American Chemical Society. 2012.

[ 3 ] a) M. Jr. Bruchez , M. Moronne , P. Gin , S. Weiss , A. P. Alivisatos , *Science* **1998** , *281* , 2013 ; b) Z. A. Peng , X. Peng , *J. Am. Chem. Soc.* **2001** , *123* , 183 .

[ 4 ] a) X. Michalet , et al., *Science* **2005** , *307* , 538 ; b) U. Resch-Genger , et al., *Nat. Methods* **2008** , *5* , 763 ; c) S. Chen , L. A. Truax , J. M. Sommers , *Chem. Mater.* **2000** , *12* , 3864 ; d) S. K. Haram , B. M. Quinn , A. J. Bard , *J. Am. Chem. Soc.* **2001** , *123* , 8860 .

[ 5 ] a) W. L. Wilson , P. F. Szajowski , L. E. Brus , *Science* **1993** , *262* , 1242 ; b) J. H. Warner , A. Hoshino , K. Yamamoto , R. D. Tilley , *Angew. Chem, Int. Ed.* **2005** , *44* , 4550 .

[ 6 ] a) Y. P. Sun , et al. , *J. Am. Chem. Soc.* **2006** , *128* , 7756 ; b) H. Peng , J. Travas-Sejdic , *Chem. Mater.* **2009** , *21* , 5563 ; c) S. N. Baker , G. A. Baker , *Angew. Chem. Int. Ed.* **2010** , *49* , 6726 ; d) Z-An. Qiao , et al., *Chem. Commun.* **2010** , *46* , 8812 ; e) X. Li , H. Wang , et al., *Chem. Commun.* **2011** , *47* , 932 .

[ 7 ] a) M. De , P. S. Ghosh , V. M. Rotello , *Adv. Mater.* **2008** , *20* , 4225 ; b) J. P. Zimmer , S.-W. Kim , S. Ohnisi , E. Tanaka , J. V. Frangioni , M. G. Bawendi , *J. Am. Chem. Soc.* **2006** , *128* , 2526 .

[ 8 ] a) J. Ito , A. Ghosh , et al. *Nature* **2002** , *417* , 452 ; b) M. A. Osta , G. K. Christophides , F. C. Kafatos , *Science* **2004** , *303* , 2030.