# DOCTORAL THESIS

## Synthesis, characterization and analytical separation of fluorescent water-soluble carbon nanoparticles

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# Synthesis, Characterization and Analytical Separation of Fluorescent Water-Soluble Carbon Nanoparticles

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**for the degree of**

**Doctor of Philosophy**

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**Hong Kong Baptist University**

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## DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

Signature: _____

Date: January 2015

## Abstract

This thesis mainly consists of two parts. The first part is concentrated on synthesizing amine/carboxylic-functionalized carbon nanoparticles (CNP) and investigating its fundamental properties in the aid of capillary zone electrophoresis (CZE) and high-performance liquid chromatography (HPLC). In this part, CNP is synthesized from citric acid (CA) and 1,2-ethylenediamine (EDA) by a microwave-assisted pyrolysis method. The resultant CNP is characterized by absorption and photoluminescence (PL) spectroscopy, transmission electron microscopy (TEM) and infrared spectroscopy (IR) to determine its overall optical properties, morphology and composition. Followed by this, the CNP product is separated and analyzed by CZE coupled with UV absorption and laser-induced fluorescence (LIF) detections. Under optimal pH and concentration of run buffer, the effect of reaction time and mole ratio of amine ($NH_2$) to carboxylic acid (COOH) moieties of the precursors on the CNP species present in CNP products are studied. Our results show that the synthesis of CNP could be improved by lengthening the microwave irradiation time and optimizing the initial mole ratio of $NH_2$/COOH in the precursors. Negatively charged CNP are obtained only when the amount of CA exceeds that of EDA, *i.e.*, the mole ratio of $NH_2$/COOH is 0.25–0.80. By contrast, when the quantity (in mole) of $NH_2$ in EDA is equal to or larger than that of COOH in CA, only positively charged and neutral CNP species are formed, inferring that the CNP species are predominantly covered by the surface-attached ammonium and amido moieties. This work highlights the merit of CZE to identify the composition of an as-prepared CNP product which is pretty much dependent on the mole ratio of $NH_2$/COOH. In addition, we carry out reversed-phase high-performance liquid chromatography coupled with fluorescence detection (RP-HPLC-FD) methodology to study the properties of each individual CNP species. Under optimal mobile phase and elution gradient

conditions, the effect of mole ratio of $NH_2$/COOH in the initial reagents on CNP product is studied. At $NH_2$/COOH = 0.67, the strongest fluorescence CNP sample is obtained. The separated CNP fractions are collected and further characterized by UV-visible absorption and PL spectroscopy, CE, TEM, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The absorption and PL emission bands ($\lambda_{em}$) of the fractions are bathochromatically shifted with the elution order of CNP on RP-HPLC. The TEM images prove that CNP are eluted from the smallest to the largest. The MALDI-TOF MS data show that CNP undergo fragmentations, closely relating to their surface-attached carboxylic acid and amide/amine moieties. This work highlights the merit of RP-HPLC coupled with fluorescence detection, TEM and MS for isolation and characterization of individual CNP species present in a CNP sample. By application of CE and HPLC separation for CNP product, better understandings of the hidden fundamental properties of CNP are achieved. The two separation techniques are well complementary to each. On one hand, CE is able to separate both positively and negatively charged CNP species which cannot be retained and separated by HPLC column, facilitating the investigation of different charge states of CNP species present in a CNP product. On the other hand, the preparative property of HPLC allows for multi-collection of the separated CNP fractions which is difficult in the case of CE analysis owning to its low sample injection volume. By using HPLC separation, the individual CNP fractions can be collected for more precious investigation of their unique photophysical and chemical properties by absorption and PL spectroscopy, TEM and MALDI-TOF MS.

The second part is focused on preparing CNP from naturally available bioresources and investigating the effect of doped heteroatoms on nitrogen (*N*) and sulfur (*S*)-doped carbon nanoparticles (*N*,*S*-CNP) based on our proposed modern RP-HPLC-FD methodology which has

been proved to be useful in separating and analyzing CNP in the first part of this work. In this part, ultrasmall *N*,*S*-CNP is prepared by microwave-assisted pyrolysis of precursors of rice as carbon source and *N*-acetyl-L-cysteine (NAC) as *N* and *S* dopants. The obtained *N*,*S*-CNP are fully characterized by elemental analysis, FTIR, x-ray photoelectron spectroscopy, TEM, UV-vis absorption and PL spectroscopy. Meanwhile, undoped CNP (derived from rice only) is also synthesized and characterized. The chemical compositions, sizes and spectral properties of undoped CNP (derived from rice only) and *N*,*S*-CNP are demonstrated to be different from each other. With the assistance of RP-HPLC-FD, the effect of different mass ratios of NAC to rice (NAC/rice) on *N*,*S*-CNP is investigated. When the NAC/rice increases from 0.20 to 0.80, the signals of the later eluted peaks increase progressively, indicating that higher NAC/rice benefits the generation of CNP with stronger fluorescence emissions. The HPLC-separated *N*,*S*-CNP fractions are collected and further characterized by MALDI-TOF MS, UV-vis absorption and PL spectroscopy, showing that the structural changes induced by doping with heteroatoms *N* and *S* plays a key role in regulating the PL properties of the *N*,*S*-CNP.

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