Analytica Chimica Acta 911 (2016) 100–107

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Elucidating the structure of carbon nanoparticles by ultra-performance liquid chromatography coupled with electrospray ionisation quadrupole time-of-flight tandem mass spectrometry

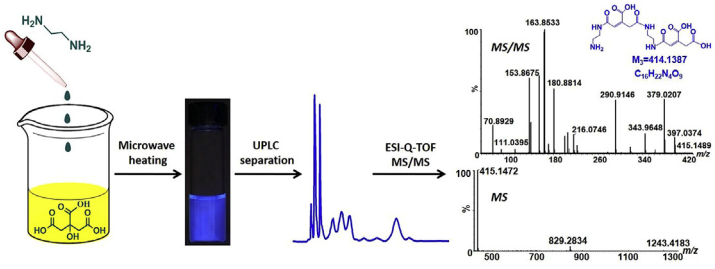
Qin Hu [a, 1], Xiangpeng Meng [a], Martin M.F. Choi [b, 2], Xiaojuan Gong [c], Wan Chan [a, *]

[a] Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, SAR, China
[b] Department of Chemistry, Hong Kong Baptist University, 224 Waterloo Road, Kowloon Tong, Hong Kong, SAR, China
[c] Research Center of Environmental Science and Engineering, and School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan, 030006, China

HIGHLIGHTS

- For the first time, UPLC-ESI-Q-TOF-MS/MS method is established to identify the chemical composition of CNP product.
- The chemical structure of each CNP species are fully characterized by high-accuracy MS and MS/MS analyses.
- The CNP species are reported to exist as non-covalent force linked supra-molecular clusters.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form
4 January 2016
Accepted 17 January 2016
Available online 27 January 2016

Keywords:
Carbon nanoparticles
ESI-Q-TOF-MS/MS
Non-covalent supramolecular clusters

ABSTRACT

A fast and accurate ultra-performance liquid chromatography coupled with electrospray ionisation quadrupole time-of-flight tandem mass spectrometry (UPLC-ESI-Q-TOF-MS/MS) method was developed for the separation and structural elucidation of fluorescent carbon nanoparticles (CNP). The CNP was synthesised from microwave-assisted pyrolysis of citric acid (CA) and 1,2-ethylenediamine (EDA). By using UPLC separation, the CNP product was well separated into ten fractions within 4.0 min. Based on high-accuracy MS and MS/MS analyses, the CNP species were revealed to display six kinds of chemical formulas, including $(C_{10}H_{20}N_4O_5)_n$, $(C_8H_{12}N_2O_5)_n$, $(C_{16}H_{22}N_4O_9)_n$, $(C_6H_8O_7)_n$, $(C_{14}H_{18}N_2O_{11})_n$, and $(C_{14}H_{16}N_2O_{10})_n$. In particular, our study revealed for the first time that the CNP species exist as supra-molecular clusters with their individual monomers units linked together through non-covalent bonding forces. These findings clearly indicated the usefulness of UPLC-ESI-Q-TOF-MS/MS in identifying the chemical composition of CNP product. It is anticipated that our proposed methodology can be applied to study the structure-property relationships of CNP, facilitating in the production of CNP with desirable spectral features.

1. Introduction

Carbon nanoparticles (CNP), which usually refer to a class of spherical carbonaceous nanomaterials with sizes below 10 nm, exhibit many excellent optical properties, such as stable photo-luminescence (PL), broad excitation wavelengths (λ_{ex}) and tunable

* Corresponding author.
E-mail address: chanwan@ust.hk (W. Chan).
[1] Postdoctoral research fellow at The Hong Kong University of Science and Technology.
[2] Present address: Acadia Divinity College, Acadia University, 15 University Avenue, Wolfville, Nova Scotia, B4P 2R6, Canada.

http://dx.doi.org/10.1016/j.aca.2016.01.034
0003-2670/

emission [1]. In striking contrast to the luminescent semiconductor quantum dots, CNP present the additional benefits of favourable biocompatibility, low cytotoxicity, easy functionalisation, good water solubility and robust chemical inertness [2–6]. Because of these dramatically superior properties, CNP have received growing interests in a wide variety of promising applications, such as bioimaging [7,8], drug delivery [9–13], therapy [14–18], optoelectronic device [19–22], and nanoprobe [23–27]. The utilisation of CNP in diverse applications requires the knowledge of a wide range of properties, the most important of which is the PL property of CNP. Although the intrinsic mechanism of CNP luminescence has not yet been clearly understood, mounting evidence suggest that the chemical structure of CNP has significant influence on the PL performance of CNP [1,28–30]. Given this information, the characterisation methods to be employed must be capable of accurately analysing the chemical structure of CNP. However, attaining this objective is beset by several challenges arising from both the instrumentation requirements and the unique nature of CNP.

The classical techniques for the structural characterisation of CNP are infrared (IR) spectroscopy [31–35], x-ray photoelectron spectroscopy (XPS) [36–40], and thermogravimetric analysis (TGA) [41–46]. These techniques certainly provide useful information about the functionalities of CNP. However, they are incapable of directly discerning the exact chemical structure and molecular formula of CNP. Mass spectrometry (MS) exhibits powerful usefulness in revealing information on the chemical structures of smaller-sized nanomaterials (<5 nm) [47]. The story of the mass spectrometric analysis of CNP is very short due to its recent arrival in the nanoparticle world. Recently, matrix-assisted laser desorption/ionisation time-of-flight MS (MALDI-TOF MS) [48,49] has been employed to determine the chemical structure of CNP. This technique provides useful information on the functionalities of CNP fractions isolated from capillary electrophoretic (CE) [48] or high-performance liquid chromatographic (HPLC) [49] separation, but exhibits lower sensitivity with increasing mass; therefore, the attempts in capturing the signals of larger ions are not easy. Besides, MALDI-TOF MS analysis cannot be directly coupled with CE or HPLC separation. For further characterisation by MALDI-TOF MS, the CNP fractions must be collected after analytical separation.

The electrospray ionisation quadrupole time-of-flight tandem mass spectrometry (ESI-Q-TOF-MS/MS), which combines the use of soft ionisation technique and sensitive MS detection, has emerged rapidly as an efficient and powerful analytical tool in investigating the assembly of oligomers as well as aggregates [50,51]. In this work, we employed ultra-performance liquid chromatography coupled with Q-TOF-MS/MS (UPLC-ESI-Q-TOF-MS/MS) to separate and characterise a CNP product synthesised from the microwave pyrolysis of citric acid (CA) and 1,2-ethylenediamine (EDA). By using UPLC separation, the complexity of the CNP product was revealed. By utilising high-accuracy MS and MS/MS analyses, two tasks-characterising chemical structures and yielding molecular formulas of CNP species-are fulfilled simultaneously. Our proposed UPLC-ESI-Q-TOF-MS/MS methodology provides valuable insights into the chemical composition of a CNP product. To the best of our knowledge, this is the first report on the characterisation of CNP by UPLC-ESI-Q-TOF-MS/MS, which certainly adds to the increasing database on the chemical characteristics of CNP.

2. Experimental

2.1. Chemicals and reagents

Anhydrous CA and formic acid (98.8%) were purchased from Fisher Scientific (Loughborough, Leics, UK). EDA was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade methanol (MeOH) was from Tedia (Fairfield, OH, USA). Ammonium acetate (NH_4Ac, 98.8%) and glacial acetic acid were from BDH Prolabo (VWR International, Leuven, Belgium). Absolute ethanol (EtOH) was from Merck KGaA (Darmstadt, Germany). Distilled deionised (DDI) water was obtained from a Millipore Milli-Q-RO4 water purification system with a resistivity higher than 18 MΩ cm (Millipore, Bedford, MA, USA). All reagents of analytical reagent grade were used as received without further purification unless noted otherwise.

2.2. Synthesis of carbon nanoparticles

Fluorescent CNP was prepared according to a published method [52] with slight modifications. In brief, 1.0000 g CA was dissolved in 10.0 mL DDI water and then mixed with 0.3126 g EDA (The mole ratio of EDA to CA is 1:1 which was reported to be the optimal condition to prepare CNP with the highest fluorescent intensity [52].) After vigorous stirring for 1.0 min, the clear transparent solution was transferred into a 50.0 mL beaker flask and heated in a domestic microwave oven (1000 W) for 4.0 min. After reaction, the beaker flask was cooled down naturally. The resultant reddish brown and foamy solid was dissolved in 4.0 mL DDI water. Then 36.0 mL anhydrous ethanol was added and centrifuged at a speed of 6000 rpm for 10.0 min to remove the excess precursors and the resulting small molecules. The deposited CNP product was dissolved in 20.0 mL DDI water and further purified by dialysing against 1.0 L DDI water through a dialysis membrane with MWCO of 500–1000 Da (Spectrum Laboratories, Rancho Dominguez, CA, USA) for 3 days with stirring and recharging with fresh DDI water every 12 h. Finally, a clear and reddish brown aqueous CNP solution was obtained and lyophilised to obtain a dry CNP product. The aqueous CNP solution showed excellent stability with no sign of precipitation for at least 3 months. For comparison, the CNP sample derived from CA only (O–CNP) was prepared according to the above preparation process.

2.3. Instrumentation

The separation and structural elucidation of the as-synthesised CNP sample were conducted by using an ACQUITY UPLC system coupled with a Xevo G2 Q-TOF mass spectrometer (Waters Corp, Milford, MA, USA). Chromatography was performed on the ACQUITY UPLC separation system, using a Waters UPLC BEH C18 column (2.1 mm × 100 mm, i.d. stainless steel) packed with 1.7 μm octadecyl (C18) bonded silica with 130 Å pore size (Milford, MA, USA). The CNP sample (0.2 mg/mL) was filtered through 0.45 μm, 13 mm i.d. cellulose acetate syringe filters (Alltech, Deerfield, IL) prior to injection. The volume of each injection was 10 μL. The mobile phase contains 10 mM NH_4Ac buffer (pH 4.0–6.0) or 0.10% formic acid (FA) in water (pH 2.8) as eluent A and MeOH as eluent B. The flow rate was 0.40 mL/min and the column temperature was maintained at 40 °C. The isocratic condtions with full aqueous mobile phase containing 10 mM NH_4Ac at pH 4.0–6.0 or 0.10% FA in water at pH 2.8 was used to study the effect of pH on separation of CNP. The isocratic condtions with mobile phase containing 0.10% FA in water (pH 2.8) and various volume percentages of MeOH (0–20%) was used to study the effect of MeOH on separation of CNP. The gradient elution program was optimised as follows: 100% A for 1.5 min, rapidly decreased to 98% A from 1.5 min to 2.0 min and maintained at 98% A from 2.0 min to 6.0 min. The gradient elution program was also used to analyse the O–CNP product.

The high-accuracy MS and MS/MS analyses of CNP were performed on a Xevo G2 Q-TOF mass spectrometer equipped with a standard ESI interface under the positive ionisation mode. Unless otherwise stated, the optimised source conditions were set as follows: capillary voltage, 2.5 kV; cone voltage, 12 V; collision energy,