International Journal of Mass Spectrometry 393 (2015) 25–33

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Exploring the ability of water soluble carbon dots as matrix for detecting neurological disorders using MALDI-TOF MS

M. Shahnawaz Khan[c], Mukesh L. Bhaisare[b], Sunil Pandey[a,d], Abou Talib[c], Shou-Mei Wu[b], Suresh Kumar Kailasa[f], Hui-Fen Wu[a,b,c,d,e,*]

[a] Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, 70, Lien-Hai Road, Kaohsiung 80424, Taiwan
[b] School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan
[c] Doctoral Degree Program in Marine Biotechnology, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan
[d] Center for Nanosciences and Nanotechnology, National Sun Yat-Sen University, 70, Lien-Hai Road, Kaohsiung 80424, Taiwan
[e] Institute of Medical Science and Technology, National Sun Yat-Sen University, 80424, Taiwan
[f] S. V. National Institute of Technology, Applied Chemistry, Gujarat, India

ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form 4 October 2015
Accepted 12 October 2015
Available online 3 November 2015

Keywords:
Carbon dots
Matrix-assisted laser desorption-ionization time-of-flight mass spectroscopy
Serotonin
Glutamic acid
Dopamine hydrochloride
2,5-Dihydroxybenzoic acid (DHB)

ABSTRACT

Carbon dots (C-dots) exhibit strong absorbance in the UV (220–350 nm) range, which was exploited to transfer the energy from N_2 laser (337 nm) of matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) to analytes for their rapid detection. Due to this strong feature and extremely small size (2–4 nm), they were used to enhance the signal intensity of MALDI-MS peaks of low molecular weight biomarkers in serum. In this study, we utilized the extraordinary property of C-dots as a matrix for the detection of serotonin (Sr), glutamic acid (GA) and dopamine hydrochloride (DA) by using MALDI-MS. These chemicals are cardinal biological indicators or biomarkers for the detection of dreadful disorders like Alzheimer's disease. The limit of detection of Sr, GA and DA was found to be 3, 5 and 8 nM, respectively. The background noises in MALDI-MS spectra can be significantly reduced by using C-dots which are superior to the conventional matrices such as 2,5-dihydroxy benzoic acid (DHB). The current approach can be further applied to many other drugs or pharmaceutical compounds in the near future.

1. Introduction

The sensitive detection and quantification of biomarkers for the life threatening diseases such as neurological disorders or cancers can make a significant contribution to theranostics. One of the pivotal contributions of nanotechnology towards this goal is to develop modern nanomaterials capable of sensing low concentration of biomarkers in body fluids, which contain plethora of interfering proteins and ions. Advances in mass spectrometry during last several decades have completely revolutionized the fields of diagnosis and treatment related with incurable neurological ailments like Alzheimer's, Parkinson's, dementia, etc. One of the most essential parameters for sensing analytes in complex biological fluids requires novel matrices which can help for better confrontation between laser as well as analytes detected by MALDI-MS [1]. It is an important tool in deciphering the rapid analysis of biomolecules. It has been used extensively in deciphering the codes of large molecules. Despite the remarkable performance of MALDI-MS, it has some limitations in analyzing low molecular weight compounds due to many background interferences by using traditional organic matrices. During the analysis, matrices play an imperative role in absorbing and transferring the energy from N_2 (337 nm) laser to analytes for effective ionization [2]. Conventional matrices such as 2,5-dihydroxy benzoic acids (2,5-DHB), sinapinic acid (SA), α-cyano-4-hydroxycinnamic acid (CHCA), picolinic acid (PA), and 3-hydroxy picolinic acid (HPA) suffer from production of fragments and clusters and sweet spots. Moreover, non-homogenous mixing of matrices with analytes often generate interferes during the analysis.

Recently, a close cousin of graphene called carbon dots (C-dots) as a matrix for MALDI-MS have been glorified to a great extent due to physical properties of self-passivation [3]. The high optical absorbance of C-dots at ~337 nm makes them a favorite choice to be used as a matrix for detecting the low molecular weight biomarkers

* Corresponding author at: Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, 70, Lien-Hai Road, Kaohsiung 80424, Taiwan.
Tel.: +886 7 5252000 3955; fax: +886 7 5253908.
E-mail address: hwu@faculty.nsysu.edu.tw (H.-F. Wu).

http://dx.doi.org/10.1016/j.ijms.2015.10.007
1387-3806/

in MALDI-MS [3]. The energy absorbed by C-dots coincides with the N_2 laser used in MALDI-MS to ionize and desorbs analytes, so sufficient laser energy can be transferred to analytes to make the C-dots as a superior matrix over the conventional matrices. In addition, C-dots have been also used intensively in drug delivery, biosensors and optical properties of biological applications [4] owing to their excellent physical–chemical properties like self-passivation, high surface area to volume ratios, good solubility in water and organic solvent [5]. In this study, we exploited the excellent properties of C-dots as a matrix for the detection and quantification of several important drugs/biomarkers [6]. Other inorganic MALDI-MS matrices such as graphitized carbon black, carbon nanotubes (CNT) [7], gold [8], graphene paper [9], fullerenes, graphene sheets [10,11] and graphite plate (laser) [12,13] are of lower anisotropic nature and stabile under high vacuum compared with the C-dots. It is also exploited for the determination of critical micelle concentration of ionic and nonionic surfactants [14]. Previously, C-dots were applied as a matrix for detecting mefenamic acid and compared with DHB matrix which is mostly used for low molecular weight compound in MALDI-MS [15]. Herein, we introduce the water-soluble C-dots for the detection of essential biomarkers (Sr, GA and DA) which are directly related with Alzheimer diseases. Sensitive and precise detection of such biomarkers can play a significant role in prognosis of the disease as well as their treatment.

DA is the most important neurotransmitter in central nervous system (CNS). It has also significant role in Parkinson's disease and drug addiction. Many researchers have tried to find the concentration of DA by using $[Fe(CN)_6]^{3-/4-}$ and reached to a concentration of 7.6×10^{-7} M [16]. GA is an important neurotransmitter as well as indicator of the Alzheimer disease. It plays an important role in brain formation, neuronal differentiation, migration and survival [17]. Stimulation of glutamate-gated ion channels causes neurological degeneration which can cause necrosis or apoptosis [18]. This may result in Alzheimer diseases, Huntington's diseases, and amyotrophic lateral sclerosis (ALS) stroke. It has been used to treat mental retardation, epilepsy, Parkinson's disease, and muscular dystrophy. Another important biomarker which is selected in our study is Sr which is a monoamine neurotransmitter popularly regarded as a feeling of peace and happiness [19]. Lack of this chemical in the human body may result in grievous psychiatric symptoms. Abnormality in serotonergic may affect the function of dorso-lateral prefrontal cortex which in the apex of cognition and emotion in the Alzheimer diseases. Because of the above important reasons, it is necessary to detect and analyze these biomarkers. Early detection is an important step in rapid diagnosis of many dreadful diseases. Proteins and other molecules can be a source of clue to find out its presence by using a promising analytical instrument called MALDI-MS. It was developed by Karas in 1988 for analysis and detection of biological samples, polymer, biomarkers and drugs in tissue analysis of fatty acids [20–22]. In this work, it is used for the detection of analyte as it enhances the soft ionization of analytes and gives significant biomarker peaks. Based on the proposed studies, the C-dots proved excellent matrix with respect to commonly used DHB matrix. C-dots as a matrix show the less background signals with high intensity of peaks. Biomarkers were quantified and spiked in different concentrations in serum by using the standard calibration curves. We also tried to explore the use of C-dots for determining the high molecular weight compounds but it shows lot of cumbersome peaks in this range, which were not reproducible. In the present work, C-dots were used as an excellent matrix for the MALDI-MS analysis in exploring the low molecular weight compounds. By using C-dots as a matrix, MALDI-MS spectra show less background or noises, sharp peaks and shot to shot reproducibility which are helpful in exploring the information of biological materials. Carboxylic acids as a functional group present on the surface of C-dots help in high water solubility, which helps in fast dispersion in making homogenous solution. Thereby, help in making excellent co-crystallization, due to small size it can easily cover the analyte which helps in getting good signals as well as sensitivity. Moreover, C-dots are easy to synthesize, have good stability, are biocompatible and cheap. These properties show that they are good candidates as an excellent matrix in analyzing low molecular weight compound.

2. Materials and methods

Acetonitrile (ACN), anhydrous citric acid and chloroform were purchased from J.T.Baker, USA. Trifluoroacetic acid (TFA) was purchased from Wako Pure Chemicals (Osaka, Japan). Serotonin (Sr), glutamic acid (GA) and dopamine hydrochloride (DA), and 2,5-dihydroxy benzoic acid (DHB) were purchased from Sigma. The water used for the experimental purpose including the cleaning of glass wares was used from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.1. Synthesis of C-dots

In ultra-pure water, 10 g of citric acid was dissolved and stirred to make a homogeneous solution. This solution was refluxed for 12 h at 60 °C till the solution turned yellowish red. Subsequently C-dots were purified by 20 mL of the solution subjected to micron-filtration using 0.45 μm nylon filter using Millipore filtration kit (Millipore, USA). Bright yellowish green solution was obtained after the filtration. Finally the powder of C-dots after vacuum drying of its solution at 120 °C was obtained. 15 mg of cysteamine hydrochloride was dissolved in 5 mL of DI water and 40 μL was measured from this stock solution and added in 1 mL of pure C-dots and total volume was adjusted to 3 mL, and was kept for 3 h under stirring. After preliminary confirmation under UV light (λ = 365 nm) to check the blue color, the solution was scrutinized by UV-Vis Spectroscopy (Perkin Elmer Lambda 25, USA) to comprehend its optical properties.

2.2. Mass spectrometric analysis

Microflex MALDI-MS (Bruker Daltonics, Germany) was used for the analysis of analyte biomarker's peaks and serum peaks. All the data were recorded triplicate in linear/positive-ion mode. MS system was operated under accelerating voltage of 20 kV and using a pulse nitrogen laser (337 nm, 4-ns pulses at 10.0 Hz, 60.0 μJ). 96-wells MALDI-MS target plate was used and MALDI-MS spectra were taken by applying 150 laser shots. The aqueous solutions of as prepared C-dots were mixed with DA, GA and Sr in equal amounts by volume (1 μL of C-dots with 1 μL of analytes). Same number of laser shots was also in consideration during the analysis on MALDI-MS plate.

2.3. Characterization

Transmission electron microscope (TEM, JEOL, and Japan) was applied for studies of shape and size of C-dots. Carbon coated grids were drop casted using dilute solution of C-dots for the sample analysis. Surface passivation of C-dots was studied using Fourier transform infrared spectrometer (Perkin Elmer, USA) after drop coating C-dots solution on KBr pellets. The UV–vis. spectra of C-dots were recorded by double beam UV-Vis spectrophotometer (Thermo, USA) over 200–800 nm wavelength ranges. Raman spectroscopy Agiltron Peak seeker of US was used. Fluorescence spectra of C-dots were acquired from fluorescence spectroscopy (Hitachi, F-2700, Japan) at various (λ_{ext} = 200, 250, 300, 350 nm) excitation values.