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CeO_2-CB nanocomposite as a novel SALDI substrate for enhancing the detection sensitivity of pharmaceutical drug molecules in beverage samples

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ABSTRACT

SALDI-MS analysis of pharmaceutical drug molecules (amitriptyline, imipramine and promazine) using carbon-based substrates, namely, activated charcoal (AC), carbon nanotubes (CNTs), carbon black (CB), graphene (rGO), graphene oxide (GO) and graphite, was explored and compared with the conventional organic matrix of MALDI. CB exhibited superior performance with respect to the other substrates in terms of detection sensitivity. Despite the effectiveness of CB to detect all drug molecules, it demonstrated a number of background signals, which may be an issue for the analysis of other molecules in the future. Therefore, for the first time, a CeO_2-CB nanocomposite was synthesized and applied as a novel SALDI substrate to minimize the background signals and stabilize CB when exposed to high laser power. The nanocomposite was characterized using XRD, TEM, FTIR, UV–Vis and N_2 sorpometry. The spectrum obtained using the novel nanocomposite in the absence of the drug molecules showed minimal background signals compared to CB. Additionally, the CeO_2-CB nanocomposite enhanced the detection sensitivity of the drug molecules with a limit of detection (LOD) of 100 ng/mL. This active substrate nanocomposite was further applied for the analysis of drug-spiked beverages without sample pretreatment or extraction, mimicking cases encountered by forensic toxicologists. All of the drugs and/or their adducts were detected in the drug-spiked beverage samples.

1. Introduction

Since the 1980s, matrix assisted laser desorption/ionization (MALDI) coupled with time of flight mass spectrometry (TOF-MS) has served as an indispensable analytical tool for the analysis of large molecules, such as peptides, proteins, polymers, polysaccharides and synthetic organic and inorganic molecules. In the MALDI technique, UV-absorbing organic acids (organic matrices) such as 2,5-dihydroxybenzoic acid (DHB), α–cyano-4-hydroxycinnamic acid (CHCA) and sinapinic acid (SA) are used to achieve high desorption and ionization efficiencies [1]. Utilizing organic matrices can produce undesired signals in the low mass region, thus influencing the analysis of small molecules. Furthermore, the suitability of the matrix during sample preparation is a matter of trial-and-error, and the presence of a “sweet spot” due to the uneven co-crystallization of the matrix and the analyte can lead to poor shot-to-shot and sample-to-sample reproducibility [2]. Therefore, surface assisted laser desorption/ionization mass spectrometry (SALDI-MS) has emerged as a powerful analytical technique for the analysis of small molecules. SALDI-MS technique substitutes the organic matrix of MALDI with active substrates, hence decreasing the interference and enabling better detection sensitivity at the low mass region.

The nascence of SALDI-MS technique was reported in 1988 by Tanka's [3] experiment in which cobalt nanopowder mixed with glycerol in organic solvents was used for the ionization of synthetic polymers and proteins. In 1995, Sunner et al. [4] used 2–15 μm graphite particles as the substrate for the analysis of peptides, proteins and low-molecular-weight analytes [5]. Since its inception, active substrates, such as nanoparticles (NPs), nanostructures, and nanocomposites, have been investigated to surmount the issues encountered with the conventional MALDI-TOF matrices [1,6,7].

There is a wide range of active substrates used for SALDI-MS applications for the analysis of small molecules [8–10]. Certain criteria must be fulfilled regarding selecting the proper SALDI-MS substrate for effective desorption/ionization and hence detection. The materials employed for SALDI-MS should exhibit high matrix/analyte loading capacity, which depends on the surface area/volume of the material, homogenous analyte distribution and sufficiently high absorption of the laser energy to ionize the analyte with minimal background interference [11]. Additionally, the surface interaction between the substrate and analyte can result in successful ionization and improved detection, a property that depends on the chemical nature of the

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analyte (for example polar or nonpolar) and the substrate [12]. Furthermore, the shape, size and morphology have also been reported to play a role in the desorption/ionization process [13–15]. Collectively, these factors have been reported to control ion generation efficiency, its desorption and subsequently its detection.

Among these substrates, carbon-based materials have attracted great attention in the forensic, environmental, clinical and biological fields. Owing to their unique properties including large surface area, low thermal conductivity, biological compatibility, versatile functionalities and low toxicity, they have been utilized as SALDI substrates for the detection of small and large molecules [16,17]. For instance, activated charcoal (AC) in its pristine state has been used for the analysis of lysine, caffeine, glucose, crystal violet and bradykinin [18]. Additionally, multiwalled carbon nanotubes (MWCNTs) have been utilized as SALDI substrate for the analysis of peptides, organic compounds, β-cyclodextrin, and N_{α}-benzoyl-$_{L}$-arginine ethylester hydrochloride, in addition to very long chain fatty acids (VLCFAs) [14,19]. Furthermore, graphene (rGO) and graphene oxide (GO) have been employed for the detection of long chain fatty acids [20], while the detection of tetradecanoic acid and 4-aminoantipyrine was achieved using graphite in an unconventional and hazardous SALDI setup [21].

Although many publications have highlighted the analysis of small molecules using carbon-based substrates, those studies have shown some shortcomings, including the presence of high background signals in the low mass region, the complexity of the setup used and the need for high laser power that can damage the substrate causing ion source contamination. Additionally, the majority of the published work has focused on particular classes of compounds such as fatty acids [22], peptides [23] and proteins [1]. However, few studies are concerned with the detection of small pharmaceutical drugs and other metabolites, which are of great interest in the medical, clinical and forensic fields.

Therefore, in this work, a group of carbon-based substrates of different size, surface chemistry, morphology, optical and physicochemical properties was used to detect small pharmaceutical drug molecules using SALDI-MS technique. The performance of these substrates was thoroughly investigated and compared under the same conditions in terms of background interference and detection sensitivity. Among the substrates, CB showed superior performance and was further used to prepare a composite of CeO_2-CB, which improved the detection sensitivity of SALDI-MS. Finally, to mimic a real case scenario encountered by forensic chemists, two types of beverages (mineral water and soft drink) were spiked with the drugs, which were then detected using the novel nanocomposite. The nanocomposite enabled the detection of all drug molecules in the samples while maintaining minimum background signals.

2. Experimental

2.1. Materials

Cerium(III) nitrate hexahydrate, sodium hydroxide, activated charcoal (AC), graphite, multiwall carbon nanotubes (MWCNTs, O. D × L 6–13 nm × 2.5–20 μm), sodium nitrate, sulphuric acid, potassium permanganate, methanol, trifluoroacetic acid (TFA), acetonitrile (ACN), amitriptyline hydrochloride, imipramine hydrochloride, promazine hydrochloride and 2,5-dihydroxybenzoic acid (DHB) were purchased from Sigma Aldrich. C65 conductive carbon black (CB) was obtained from MTI Corporation.

2.2. Characterization

The morphology of the carbon-based substrates was investigated using field emission scanning electron microscope (FESEM) model (LEO SUPRA 50VP). The optical properties of the carbon-based substrates and CeO_2-CB nanocomposite were determined using UV–Vis spectra and were recorded using Agilent Cary 5000 UV–Vis spectrometer. The chemical composition was characterized by Fourier transform infrared spectrometer (FTIR), and the spectra were recorded on a Jasco 6300 FTIR in the range of 400–4000 cm^{-1}. For the CB and CeO_2-CB nanocomposite, N_2 adsorption-desorption isotherms were determined at − 195 °C using a model Gemini VII, ASAP 2020 automatic Micromeritics sorptometers (USA) equipped with an out-gassing platform. The bulk and surface characteristics were determined by evaluating their X-ray powder diffraction (XRD) patterns, obtained by using Bruker's D8 ADVANCE diffractometer with Cu Kα (λ = 0.154 nm) radiation under 40 kV, 40 mA and a scanning range of 10–80° 2θ. The morphology was determined using transmission electron microscopy (TEM) with a JEOL JEM 1230 (JEOL Ltd., Japan) operating at 120 kV.

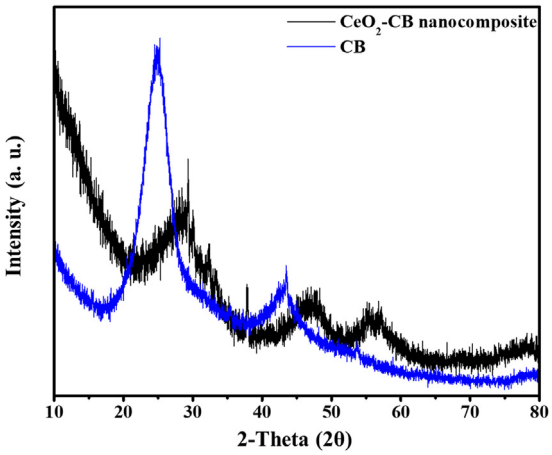

Fig. 1. XRD spectra of CB and CeO_2-CB nanocomposite.

2.3. Synthesis of GO, rGO and CeO_2-CB nanocomposite

rGO and GO were synthesized according to a modified Hummers's method [24] (see supporting information). The CeO_2-CB nanocomposite was prepared in a single-pot reaction by dispersing 0.1 g of CB in 100 mL of 0.4 M NaOH at room temperature. To this, 50 mL of 0.03 M $Ce(NO_3)_3{\cdot}6H_2O$ was added dropwise under vigorous stirring and was left to mix for 4 h. The precipitate was then washed with water and dried in the oven at 90 °C for 5 h.

2.4. Sample preparation for SALDI/MALDI-MS analysis

A saturated solution of DHB in ACN/H_2O (2:1, v/v) containing 0.1% TFA was prepared as a model organic matrix of MALDI. SALDI substrates were prepared as follows: 10 mg of AC, CNTs, CB, GO, rGO, graphite and CeO_2-CB nanocomposite were dissolved in 1 mL of methanol, separately. Each sample was prepared using the dried-droplet method to achieve homogeneous distribution. Equal volumes of the analytes (1 mg/mL in methanol) and matrix/substrate were mixed vigorously, then 2 μL of the mixture were deposited on the target plate. The mixture was allowed to dry at room temperature, after which the target plate was introduced into the mass spectrometer.

2.5. SALDI/MALDI-MS analysis

SALDI/MALDI-MS analysis was performed using the Bruker ultrafleXtreme MALDI-TOF/TOF-MS system equipped with smartbeam-