Analytica Chimica Acta 802 (2013) 103–112

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Loading of free radicals on the functional graphene combined with liquid chromatography–tandem mass spectrometry screening method for the detection of radical-scavenging natural antioxidants

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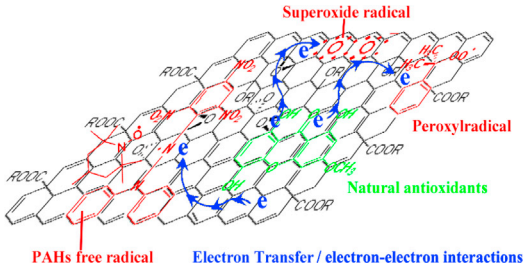
HIGHLIGHTS

- Free radical-LC/MS for detection of radical scavenging antioxidant was developed.
- LC/MS shows good performance for the detection of radical scavenging antioxidants.
- Radical-LC/MS screening was used for evaluating the radical scavenging capacity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 July 2013
Received in revised form 6 September 2013
Accepted 18 September 2013
Available online 30 September 2013

Keywords:
Antioxidants
Loading of free radicals
Radical-scavenging
Functionalized graphene
LC–MS
Free radical reaction kinetics

ABSTRACT

A novel free radical reaction combined with liquid chromatography electrospray ionization tandem mass spectrometry (FRR-LC–PDA-ESI/APCI-MS/MS) screening method was developed for the detection and identification of radical-scavenging natural antioxidants. Functionalized graphene was prepared by chemical method for loading free radicals (superoxide radical, peroxyl radical and PAHs free radical). Separation was performed with and without a preliminary exposure of the sample to specific free radicals on the functionalized graphene, which can facilitate reaction kinetics (charge transfers) between free radicals and potential antioxidants. The difference in chromatographic peak areas is used to identify potential antioxidants. The structure of the antioxidants in one sample (*Swertia chirayita*) is identified using MS/MS and comparison with standards. Thirteen compounds were found to possess potential antioxidant activity, and their free radical-scavenging capacities were investigated. The thirteen compounds were identified as 1,3,5-trihydroxyxanthone-8-O-β-D-glucopyranoside (PD1), norswertianin (PD2), 1,3,5,8-tetrahydroxyxanthone (PD3), 3, 3′, 4′, 5, 8-penta hydroxyflavone-6-β-D-glucopyranosiduronic acid-6′-pentopyranose-7-O-glucopyranoside (PD4), 1,5,8-trihydroxy-3-methoxyxanthone (PD5), swertiamarin (PS1), 2-C-β-D-glucopyranosyl-1,3,7-trihydroxylxanthone (PS2), 1,3,7-trihydroxylxanthone-8-O-β-D-glucopyranoside (PL1), 1,3,8-trihydroxyl xanthone-5-O-β-D-glucopyranoside (PL2), 1,3,7-trihydroxy-8-methoxyxanthone (PL3), 1,2,3-trihydroxy-7,8-dimethoxyxanthone (PL4), 1,8-dihydroxy-2,6-dimethoxy xanthone (PL5) and 1,3,5,8-tetramethoxydecussatin (PL6). The reactivity and SC_{50} values of those compounds were investigated, respectively. PD4 showed the strongest capability for scavenging PAHs free radical; PL4 showed prominent scavenging capacities in the lipid peroxidation processes; it was found

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http://dx.doi.org/10.1016/j.aca.2013.09.041

that all components in *S. chirayita* exhibited weak reactivity in the superoxide radical scavenging capacity. The use of the free radical reaction screening method based on LC–PDA-ESI/APCI-MS/MS would provide a new approach for rapid detection and identification of radical-scavenging natural antioxidants from complex matrices.

1. Introduction

Scientists increasingly believe free radicals play a major role in the development of aging-related diseases, such as Parkinson's, inflammatory disorder, cardiovascular diseases, cancer, arthritis, Alzheimer's and premature aging [1–9]. Free radicals are formed during oxidation processes occurring in living organisms, which are known to be responsible for health damage and accelerated aging [10,11]. There is also a considerable amount of free radicals in the air, especially contaminated environment. One example is polycyclic aromatic hydrocarbons (PAHs) in polluted environment. PAHs that contain three or more aromatic rings can absorb UVA (320–400 nm) and visible (>400 nm) light. The resulting excited PAHs can act as sensitizers to transfer energy or one electron to molecular oxygen and other molecules to generate free radicals. Metabolism of PAHs through one electron oxidation resulting in reactive free radical intermediates that can react with DNA to form DNA covalent adducts has also been reported. PAHs free radicals can damage cellular constitutes such as cell membranes, nucleic acids, or proteins, resulting in acute toxicity and genotoxicity including carcinogenicity, mutagenicity and teratology [12–20].

It is believed that antioxidants can protect our bodies from free radical damage and thus play a central role in the prevention of age-related diseases. As a result, there has been an increasing interest in naturally occurring antioxidants [21–25]. In the detection of radical-scavenging natural antioxidants, the main mechanism of the reaction between free radicals and bioactive molecules is charge-transfer [2,12–15,26–29], namely that bioactive molecules can quench free radicals by hydrogen atom transfer (HAT), single electron transfer (SET), radical adduct formation (RAF), or sequential proton loss electron transfer (SPLET) (see Supporting Information Fig. S1). However, in the detection and identification of the major antioxidants in biological samples by use of free radical spiking test, the reactions between free radicals and potential bioactive molecules are inhibited because of the higher charge-transfer resistance and interference of matrices. Therefore, most developed screening methods for the detection of radical-scavenging natural antioxidants from complex matrices sometimes fail to detect the free radical-scavenging activity of active ingredients.

In the search for new natural antioxidants, complex mixtures are frequently encountered. Availability of a simple, reliable and rapid method for separation and screening of potential antioxidants from complex mixture is essential. Previous methods involving online antioxidant assays coupled with HPLC were successfully developed [30–35]. MS has also previously been used to elucidate the structure of antioxidants in various samples [24,36,37]. The online HPLC assays have been widely used for stable radicals, including DPPH and ABTS free radicals, and acidic potassium permanganate chemiluminescence detection [38]. At present, the use of active radicals, including $^{\bullet}OH$, $RO^{\bullet}$, $O_2^{\bullet -}$, $ROO^{\bullet}$, $^{\bullet}NO$, *etc.*, shows limitations to the online HPLC assays because the active radicals have a very short half-life (t½, approximately several seconds to 10^{-12} s).

Graphene is a two-dimensional sheet of carbon atoms bonded by sp^2 hybrid orbitals. This structural characteristic is the reason for the extraordinary properties of graphene, which include a very large surface area, low electrical resistance, outstanding charge-transfer characteristics and high chemical stability. Functionalized graphene consisted of intact graphitic regions interspersed with sp^3-hybridized carbons containing carboxyl and epoxide functional groups on the edge, top, and bottom surfaces of each sheet and sp^2-hybridized carbons on the aromatic network [39–43]. The Transmission electron microscope (TEM) image and chemical structure of functionalized graphene prepared by chemical method are shown in Fig. 1.

The electronic properties in functionalized graphene are due to delocalized π bonds above and below the basal plane [44]. The large π conjugated structure of functionalized graphene can form π–π stacking interaction. The functionalized graphene with large specific area has good dispersion in different polar solutions (including water, methanol, ether, *etc.*) due to the intrinsic chemical structure composed of a hydrophobic backbone and hydrophilic side chains. Therefore, the functionalized graphene can act as a loading substrate of free radicals, which can promote charge transfer in complex matrices (See Fig. 2).

Swertia chirayita, as an antioxidant natural plant [45], is a medicinal plant indigenous to temperate Himalaya. The plant is used

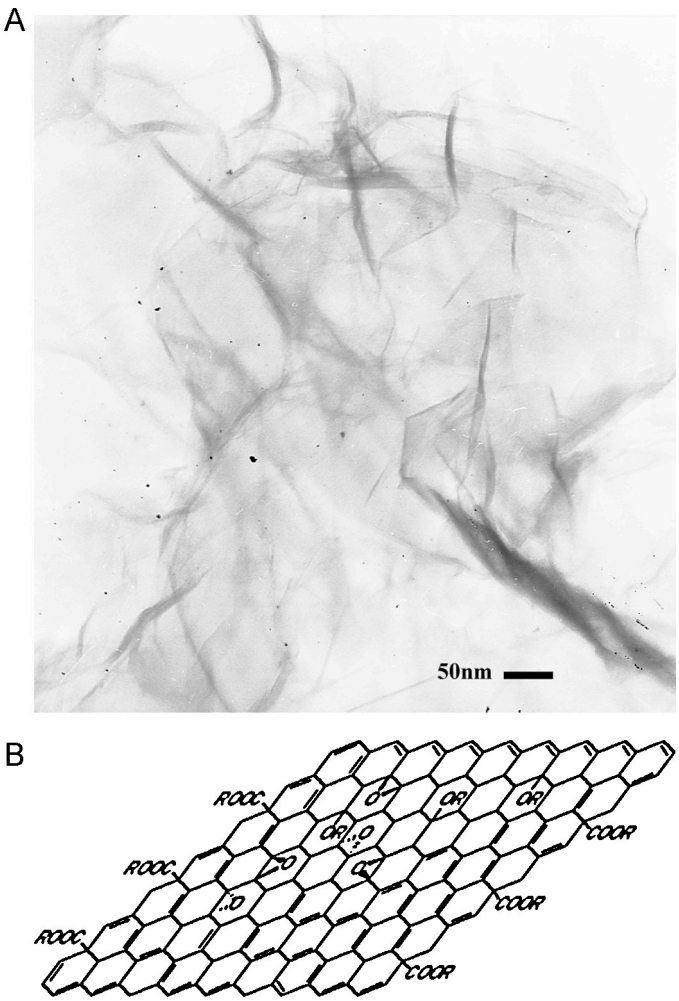

Fig. 1. The transmission electron microscope (TEM) image and chemical structure of functionalized graphene.