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Multi-walled carbon nanotube-impregnated agarose film microextraction of polycyclic aromatic hydrocarbons in green tea beverage

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ABSTRACT

A new microextraction procedure termed multi-walled carbon nanotube-impregnated agarose film microextraction (MWCNT–AFME) has been developed. The method utilized multi-walled carbon nanotubes (MWCNTs) immobilized in agarose film to serve as adsorbent in solid phase microextraction (SPME). The film was prepared by mixing the MWCNTs in agarose solution and drying the mixture in oven. Extraction of selected polycyclic aromatic hydrocarbons was performed by inserting a needle through circular MWCNT-impregnated agarose films (5 mm diameter) and the assembly was dipped into an agitated sample solution prior to micro high performance liquid chromatography–ultraviolet analysis. Back extraction was then performed using ultrasonication of the films in 100 μL of solvent. The film was discarded after single use, thus avoiding any analyte carry-over effect. Due to the mesoporous nature of the agarose film, the MWCNTs were immobilized easily within the film and thus allowing for close contact between adsorbent and analytes. Under the optimized extraction conditions, the technique achieved trace LODs in the range of 0.1 to 50 ng L^{-1} for the targeted analytes, namely fluoranthene, phenanthrene and benzo[a]pyrene. The method was successfully applied to the analysis of spiked green tea beverage samples with good relative recoveries in the range of 91.1 to 107.2%. The results supported the feasibility of agarose to serve as adsorbent holder in SPME which then minimizes the consumption of chemicals and disposal cost of organic wastes.

1. Introduction

Miniaturization has become an important trend in the development of sample preparation techniques due to the concern of environment pollution. Aware of the pollution issue, environmental friendly practices and green analytical chemistry have been incorporated into research with the aim to reduce the use of hazardous chemicals.

Solid phase microextraction (SPME) is a solventless technique developed by Arthur and Pawliszyn [1]. The advantages and drawbacks of SPME essentially originated from the same source, where the SPME extract can be injected directly into chromatography but excessive device must be attached or modified at the inlet of the instruments. Aware of the insufficient instrument and excessive device required for the SPME analysis, the use of stir bar sorptive extraction (SBSE) [2,3], microextraction in packed syringe (MEPS) [4], solid phase membrane tip extraction (SPMTE) [5], membrane protected adsorbent SPME [6] and adsorbent film SPME [7–9] have been reported to simplify the lab procedure, where the back extraction was carried out in solvent with the aid of either temperature, ultrasonication or electric force. The modified or enhanced SPME techniques are not solventless but have greatly reduced the organic solvents and waste.

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants identified as mutagen or carcinogen since 1976 [10]. Green tea is one of the popular beverages widely consumed by the world's population. Green tea with its well known antioxidant properties has led to the realization of its importance in anti-mutagenic and anticlastogenic treatments [11]. However, the contamination of PAHs in vegetation [12–14] from gaseous and particle-bound PAHs was frequently reported and this included tea leaves [15,16]. It was reported that factors such as tea variety, tea/water ratio, brewing time, washed tea or unwashed tea, and covered-up or uncovered-cup tea during tea manufacturing process significantly affect the transfer of PAHs to end product [17]. Therefore, determination of PAHs residues in green tea is important in order to prevent adverse effect to drinking green tea and to assist in the establishment of the maximum residue limits. Solid phase extraction (SPE) [18] and liquid–liquid extraction

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(LLE) followed by column chromatography clean-up [15,19] have been extensively applied to extract PAHs in tea. However, both techniques consume large amounts of organic solvents and chemicals and thus they are less supportive towards green chemistry. Lately, micro-scale sample preparation techniques have emerged to address this shortcoming. SBSE [20] offered trace detection limit with longer extraction time that exceeded one hour. First generation headspace SPME [21] required excessive injection port at instrument which might not be convenient for routine analysis although the technique gave detection limits as low as SBSE with shorter extraction time. In short, the application of micro-scale sample preparation techniques to extract PAHs from tea is still limited.

Agarose, a polysaccharide extracted from seaweed [22], is quoted as green polymer due to its biodegradable nature. Due to its hydrophilic, gelling and inert properties, agarose has been extensively studied and applied as a medium in gel electrophoresis, template to control the structural properties, drug delivery agent and optical sensor supporting material [22–25]. Lately, agar–agar powder has been modified and employed as sorbent in SPE for the extraction of mercury in water and fish samples [26]. A simple approach termed agarose film liquid phase microextraction has been demonstrated for the extraction and preconcentration of PAHs from environmental water samples [27]. In this report, agarose film was utilized as a barrier between organic extractant and sample solution. This approach has a double "green chemistry" nature due to its microextraction format and the biodegradability of the agarose film.

Carbon nanotubes (CNTs) with tubular structure of carbon atom sheets were discovered by Iijima [28]. Since then, CNTs have become multidisciplinary research focus due to its unique mechanical, electrical, chemical and thermal properties [29]. Recently, the application of CNTs as sorbents in separation science has gained considerable interest, especially for the extraction and pre-concentration of targeted analytes. The application of multi-walled carbon nanotubes (MWCNTs) as adsorbent in SPE has resulted in superior enrichment of PAHs [30] and triazines [31] from water samples. Basheer and co-workers demonstrated porous membrane protected MWCNT micro-SPE for the extraction of organophosphorus pesticides from sewage sludge samples. The technique provided cost effective and selective extraction which eliminated the filtration step [6]. Recently, Pardasani et al. [32] reported the application of magnetic MWCNTs assisted dispersive SPE that was simple, rapid and offered higher recoveries of nerve agents and their markers from muddy water as compared to SPE that utilized C_{18} as sorbent. The high surface area of MWCNTs has been beneficial to the effective mass transfer of analytes [32].

In this work, multi-walled carbon nanotube-impregnated agarose film microextraction (MWCNT-AFME) system is proposed for the first time and applied to the analysis of PAHs in green tea beverage. Mesoporous agarose film was employed as adsorbent holder and MWCNTs were utilized as adsorbent and immobilized within the agarose film. Circular-shaped MWCNT-impregnated agarose film was pierced by a needle and dipped into the sample solution for extraction. This innovation utilizes biodegradable agarose to serve as adsorbent holder in SPME and contributes to minimize the use of chemicals and organic disposal.

2. Experimental

2.1. Chemicals and materials

Phenanthrene (PHE), fluoranthene (FLA) and benzo[a]pyrene (BaP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions (200 mg L^{-1} of each analyte) were prepared by dissolving in acetonitrile (PHE and BaP) and methanol (FLA). Working standard solutions were prepared weekly using methanol from stock standard solutions. All standard solutions were stored in dark at 4 °C when not in use. Tetrahydrofuran (THF), isopropyl alcohol (IPA), methanol (MeOH) and acetonitrile (ACN) were HPLC grade purchased from Merck (Darmstadt, Germany). Agarose (molecular grade) was obtained from Promega (Madison, USA). MWCNTs (specific surface area >233 m^2 g^{-1}, purity $>95\%$, 8–15 nm outer diameter $\times 50$ μm in length) were purchased from Sun Nanotech (Jiangxi, China). A hot plate stirrer (Corning, USA) and a stirring bar (12×4 mm) were used to agitate the samples during extraction.

2.2. Chromatographic conditions

All analyses were performed using a micro high performance liquid chromatography (μ–HPLC) (Agilent Technologies, Milan, Italy) coupled with a ultraviolet detection (Agilent Technologies). The chromatographic separation of PAHs was carried out on a ZORBAX Eclipse Plus C_{18} column (2.1×100 mm, 3.5 μm) from Agilent. The separation was performed using isocratic mobile phase ACN-water (80:20) (v/v) at column temperature of 25 °C. The flowrate, injection volume and detection wavelength were fixed at 0.2 mL min^{-1}, 2 μL and 254 nm, respectively. Chromatographic data were processed using Agilent Chemstation software.

2.3. Preparation of multi-walled carbon nanotube-impregnated agarose film (MWCNT-AF)

Agarose (0.30 g) was added with 30 mL of deionized water (Millipore, France) and mixed. The content was brought to boil to completely dissolve the agarose. MWCNTs (90 mg) were added into the boiled agarose solution and stir to mix well. An aliquot of the warm solution (4.0 mL) was pipetted into a glass Petri dish (50 mm in diameter) and the solution was allowed to cool and gel at room temperature for at least 30 min. The Petri dish was dried in an oven at 40 °C for 24 h. The MWCNT-AF formed was punched into circular pieces with certain size (5 mm diameter) with a puncher.

The film obtained was then sent for subsequent characterization using Hitachi S-4800 field emission scanning electron microscope (FESEM) (Tokyo, Japan) and nitrogen adsorption method. FESEM was used to analyze the morphology of the agarose film whereas Nitrogen adsorption ASAP 2010 Micromeritics surface analyzer (Norcross, GA, USA) was used to obtain Brunauer Emmett Teller (BET) surface area of the film.

2.4. Multi-walled carbon nanotube-impregnated agarose film microextraction (MWCNT-AFME)

Water sample (20 mL) was pipetted into a 25-mL sample vial and a magnetic stirrer was placed into the sample. Hypodermic needle was used to pierce the Parafilm and then pieces (1–4) of the MWCNT-AF, alternately separated by silicone septum. The assembly was then dipped into IPA for 2 min followed by deionized water for 1 min to condition the films before dipping it into the sample solution for extraction. The sample vial was sealed immediately with the Parafilm (Fig. 1). After stirring at a speed of 800 rpm for 40 min, the films were removed and sonicated with 100 μL of THF for 15 min. The THF was filtered through 0.2 μm nylon syringe filter prior to μ–HPLC analysis.