Journal of Chromatography A, 1214 (2008) 11–16

Contents lists available at ScienceDirect

Journal of Chromatography A

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

On-line flow injection-cloud point preconcentration of polycyclic aromatic hydrocarbons coupled with high-performance liquid chromatography

Cheuk Fai Li[a], Jonathan W.C. Wong[b], Carmen W. Huie[a,1], Martin M.F. Choi[a,*]

[a] Department of Chemistry, Hong Kong Baptist University, 224 Waterloo Road, Kowloon Tong, Hong Kong SAR, China
[b] Department of Biology, Hong Kong Baptist University, 224 Waterloo Road, Kowloon Tong, Hong Kong SAR,China

ARTICLE INFO

Article history:
Received 5 August 2008
Received in revised form 8 October 2008
Accepted 15 October 2008
Available online 21 October 2008

Keywords:
Cloud point extraction
Preconcentration
Soil
Polycyclic aromatic hydrocarbons

ABSTRACT

Cloud point methodology has been used to develop a novel preconcentration and an analytical method for polycyclic aromatic hydrocarbons (PAHs) in soil sample. The nonionic surfactant Tergitol 15-S-7 was successfully used as the surfactant-mediated extractant in both ultrasonic and microwave-assisted extractions. Over 90% of recoveries for various PAHs in soil were obtained under the optimal experimental conditions. The extracts were then preconcentrated and analyzed by our proposed on-line coupling method “flow injection-cloud point preconcentration-high-performance liquid chromatography” (FI-CPP-HPLC) equipped with a fluorescence detector and an excitation/emission wavelength program. The preconcentration system and optimal working conditions were established. The limit of detection of the FI-CPE-HPLC system ranges from 0.101 to 0.456 μg/L for the selected PAHs, *i.e.*, phenanthrene, pyrene, chrysene, benzo[*k*]fluoranthene and benzo[*a*]pyrene. Our proposed technique provides a reliable, simple and automatic analytical method for the determination of PAHs in environmental soil samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large class of compounds that contain multiple benzene rings. These compounds are flat and each carbon has three neighboring atoms much like graphite. Since they are very stable in nature and some of them are highly carcinogenic and mutagenic [1,2], much attention has been paid to monitor and separate PAHs in recent years. Some common PAHs are naphthalene, fluorene, phenanthrene (PHE), anthracene, fluoranthene, pyrene (PYR), benzo[*a*]anthracene, and benzo[*a*]pyrene (BAP). It is well-known that BAP is highly carcinogenic among different PAHs. The maximum acceptable concentration level for most PAHs is usually 0.2 μg/L; however, for BAP, it is 0.02 μg/L [3]. As such, the development of sensitive analytical technique for PAHs is of particular importance. PAHs are formed by incomplete combustion of coal oil, gas, wood, garbage, or other organic substances [4]. Burning of plants or vegetation will release PAHs naturally, but burning of fossil fuels is one of the significant sources of PAHs to the environment especially in cities with large population. PAHs are considered as very significant environmental pollutants. In fact, they can be found in a wide variety of environmental samples including soil, sediment, air, and rivers. They are considered to be the priority pollutants by both the European Environmental Agency and the US Environmental Protection Agency [5].

Traditionally, solid samples of different matrices are extracted with organic solvents such as hexane/acetone mixture by Soxhlet extraction. A basic Soxhlet extraction apparatus consists of a solvent reservoir, a heat source and a water-cooled reflux condenser. However, this method is rather slow, typically more than 24 h of extraction, and large quantity of solvent is consumed [6].

In the present work, we study the micellar extraction of PAHs in environmental soil samples and also the application of cloud point (CP) phenomenon to concentrate various PAHs on a preconcentration column. Two different extraction techniques, ultrasonic and microwave-assisted extractions, coupled with a micellar system have been studied and compared. Five probe PAHs including PHE, PYR, chrysene (CHR), benzo[*k*]fluoranthene (BKF) and BAP were chosen in our proposed study. These PAHs represent “light”, “medium” and “heavy” PAHs out of their 16 counterparts. Generally, all these five PAHs possess typical properties of common PAHs and thus can simulate real environmental situation. The main objective of this work is to develop a fully automatic system to couple cloud point preconcentration (CPP) with an HPLC system. PAHs were initially concentrated on a preconcentration column after ultrasonic and microwave-assisted extractions. Such preconcentration is crucial and necessary since the concentration of PAHs in most environmental soil samples is generally very low, typically from μg/kg to ng/kg level [7]. The CP phenomenon is well-known and Watanabe firstly employed this phenomenon as an extrac-

---

[*] Corresponding author. Tel.: +852 3411 7839; fax: +852 3411 7348.
E-mail address: mfchoi@hkbu.edu.hk (M.M.F. Choi).

[1] Deceased in December 2006.

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.10.062

tion technique [8] in 1978. Previous researches show that cloud point extraction (CPE) can be used to extract various organic compounds and it is usually used as a preconcentration step prior to chromatographic separation and detection [9–13]. Sicilia et al. [14] successfully applied acid-induced CP method to extract PAHs from environmental samples using an anionic surfactant. Pino et al. [15] developed a preconcentration method using nonionic surfactant before high-performance liquid chromatographic (HPLC) analysis. Ferrer et al. [3] developed a preconcentration method plus a clean-up procedure to remove any surfactant which may affect chromatographic analysis. However, all of these similar preconcentration methods require a lot of manual procedures. Such approaches are often complicated, time-consuming and are difficult to realize automation.

Fang et al. [16] first demonstrated the on-line incorporation of CPE to flow injection analysis. As such, CPE can be performed in a continuous flow system as compared to the several manual operation procedures in conventional method. This research group achieved several attributes such as inducing CP phenomenon, separation of surfactant-rich phase from the aqueous phase, and detecting trace amount of analyte in the presence of highly scattering surfactant medium. Later studies were carried out to explore other applications of on-line CPE system. Soong et al. [17] used an on-line flow injection (FI)-CPE to analyze BAP in water which demonstrates the possibility of on-line CPE of PAHs. Unfortunately, these on-line methods can only analyze one type of PAHs at one time and they were easily interfered by impurities.

Surfactant plays a pivotal role in a CPE process. Therefore, proper choice of surfactant is important to the success of CPE. A nonionic surfactant Tergitol 15-S-7, a secondary ethoxylated alcohol, has been successfully employed to CP extract some selected PAHs in aqueous solutions [18,19]. In this work, this nonionic surfactant was selected for “flow injection-cloud point preconcentration-high-performance liquid chromatography” (FI-CPP-HPLC) analysis of PAHs because of its several advantages. First, it is a nonionic surfactant so that the CP can be salt-induced. Second, Tergitol 15-S-7 has a very low CP of 20–42 °C which is more convenient for operation. Third, it has very weak UV (>260 nm) absorption and thus fluorescence interference on PAHs detection is minimized (see below). Finally, this surfactant is biodegradable and is accepted as a general-purpose cleaner by the US Department of Agriculture [19,20]. As such, the determination of PAHs can be conveniently performed by HPLC with fluorescence detection.

The objective of this work is to develop an on-line FI-CPP-HPLC system that can preconcentrate, separate and analyze PAHs in one single operation unit. A conventional Soxhlet extraction method followed by GC–MS analysis was performed for comparison purposes. Our proposed method has been successfully applied to the determination of PAHs in environmental soil samples.

2. Experimental

2.1. Chemicals

Ammonium sulfate, PHE, PYR, CHR, BKF, BAP, and Tergitol 15-S-7 were purchased from Aldrich (Milwaukee, WI, USA). HPLC-grade acetonitrile (ACN), diethyl ether, *n*-hexane, acetone, and methanol were supplied by Labscan Asia (Bangkok, Thailand). All aqueous solutions were prepared with distilled deionized (DDI) water from a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Apparatus

The FI-CCP system consisted of two MasterFlex C/L peristaltic pumps, silicone tubing, Tygon tubing, PTFE tubing (Cole-Parmer Instrument, Vernon Hills, IL, USA), and a laboratory-made preconcentration column. The preconcentration column was constructed from a stainless steel tube (30 mm long, I.D. 3.2 mm) packed with 0.03 g DAR-7-R83 silica gel (Riedel-de Haën, Seelze, Germany). The HPLC instrument included an HP1100 series binary pump, a degasser, and a HP1046A programmable fluorescence detector (Hewlett-Packard, Santa Clara, CA, USA). The analytical column was an Econosphere 5-μm C18, 250 mm × 3.2 mm column from Alltech (Brentwood, TN, USA). A personal computer equipped with an HP Chemstation program for LC system was used to acquire and store the chromatographic data. Ultra-sonication extraction was conducted on a Branson 2510 sonicator (Cole-Parmer) thermostated at 50 °C by a water-bath. Microwave-assisted extraction was performed on a CEM MARS 5 microwave digestion system (Matthews, NC, USA). An Agilent 6890N network GC system equipped with an Agilent 5973 mass selective detector (Santa Clara, CA, USA) was employed to perform GC–MS analysis. All samples were filtered by 0.45-μm nylon syringe filters (Filtrex, Attleboro, MA, USA) prior to chromatographic analysis.

2.3. Cloud point phenomenon study

The CP phenomenon of Tergitol 15-S-7 was studied by the following method. The stock surfactant was diluted as volume percentage (v/v) by DDI water. The concentration of surfactant varied from 0.05 to 4.0%. Then the mixtures were heated and the temperatures were recorded. When the solution became turbid, the temperature at that moment was known as CP. The effect of salt on the CP of 1.50% Tergitol 15-S-7 solution was also investigated. Different concentrations of salt solutions in 1.50% Tergitol 15-S-7 solutions were prepared. Then their CPs were observed by the aforementioned method. Ammonium sulfate was used as it is reported to be the best type of salt to induce CP [17].

2.4. Soil treatment

Soils for both ultrasonic and microwave-assisted extractions were collected at Tai Lung Experimental Farm (Fan Leng, Hong Kong, China). After sieving, soil fractions with a particle size of 250 μm (USA Standard Test Sieve, ASTM specifications No. 60) were selected. Since the PAHs level in the soil samples were very low, they were spiked with known standards of PAHs for FI-CPP-HPLC analysis. Standard solid PAHs were dissolved in ACN and were added into the soil samples in a glass round-bottom flask. The flask was shaken for 6 h to ensure that the standards were evenly distributed in the soil samples. Afterwards, the soil samples were dried to remove the solvent by a stream of nitrogen. The soil with the flask was stored in a dry box for future use. The concentrations of spiked PAHs were 10 μg in 1 g of soil [21].

2.5. Ultrasonic extraction

0.05–0.1 g of the spiked soil was put into a 40 mL capped glass bottle. Various concentrations of Tergitol 15-S-7 solutions (0.05–4.00%) were added and the bottle was tightly closed. Then the bottle was sonicated for 120 min at 50 °C and the extract was filtered through a 0.45-μm syringe filter. The resulting extract was analyzed by the FI-CPP-HPLC system.

2.6. Microwave-assisted extraction

0.05–0.1 g of the spiked soil was loaded into a microwave extractor. Various concentrations of Tergitol 15-S-7 solution (0.05–4.00%) were added. The vessel was tightly closed and positioned into the microwave extractor. The power was set at 600 W, the pressure was