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Room temperature fluorescence spectroscopy of benzo[*a*]pyrene metabolites on octadecyl extraction membranes

Bassam Alfarhani [a], Maha Al-tameemi [a], Agustina V. Schenone [b], Hector C. Goicoechea [b], Fernando Barbosa Jr. [c], Andres D. Campiglia [a,*]

[a] Department of Chemistry, University of Central Florida, 4000 Central Florida Blvd, Physical Sciences Room 255, Orlando, FL 32816-2366, United States
[b] Universidad Nacional del Litoral, CONICET, FBCB, Laboratorio de Desarrollo Analítico y Quimiometría (LADAQ), 3000 Santa Fe, Argentina
[c] Laboratório de Toxicologia e Essencialidade de Metais, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo 14.040-903, Brazil

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ABSTRACT

Benzo[*a*]pyrene (B[*a*]P) is a prototypic carcinogenic polycyclic aromatic hydrocarbon (PAH), which requires metabolic activation to produce its detrimental effects. Measurement of B[*a*]P metabolites in human urine could provide a direct way to assess individual differences in susceptibility to PAH-related cancer. This article focuses on the development of screening methodology for the routine analysis of B[*a*]P metabolites in urine samples. It explores the solid-surface room-temperature fluorescence (RTF) properties of 3-hydroxy-benzo[*a*]pyrene, benzo[*a*]pyrene-*trans*-9,10-dihydrodiol, benzo[*a*]pyrene-r-7,t-8,c-9-tetrahydrotriol and benzo[*a*]pyrene-r-7,t-8,c-9,c-10-tetrahydrotetrol previously extracted from urine samples with octadecyl-silica membranes. Relative standard deviations varying from 2.1% (benzo[*a*]pyrene-r-7,t-8,c-9-tetrahydrotriol) to 8.6% (3-hydroxy-benzo[*a*]pyrene) are obtained with the aid of fiber optic probe that eliminates the need for manual optimization of signal intensities. Analytical recoveries from human urine samples varied from 87.5 ± 3.1% (3-hydroxy-benzo[*a*]pyrene) to 99.8 ± 2.5% (benzo[*a*]pyrene-r-7,t-8,c-9,c-10-tetrahydrotetrol). The excellent analytical figures of merit and the simplicity of the experimental procedure demonstrate the potential of this approach for screening biomarkers of PAH exposure in numerous urine samples.

1. Introduction

Considerable efforts have been made to improve luminescence measurements of compounds adsorbed on solid substrates [1,2]. As a result, solid surface luminescence analysis is a widely accepted tool in environmental, pharmaceutical, food and agricultural science [3]. Particularly attractive is the use of solid-phase extraction (SPE) membranes for the on-site analysis of polycyclic aromatic compounds in water samples [4–8]. Attractive features include a simple experimental procedure and compatibility with portable instrumentation. Due to the non-destructive nature of luminescence measurements, extraction membranes can be brought to the lab for subsequent sample elution and fluorophore confirmation via high-resolution techniques [9–12].

Solid surface room temperature fluorescence (RTF) spectroscopy on SPE membranes was first proposed for the analysis of polycyclic aromatic hydrocarbons (PAHs) in water samples [5,13]. Octadecyl silica membranes were cut into tabs and subsequently immersed into aqueous solution for PAHs extraction. Depending on the PAH, the extraction step varied from one to two hours of immersion time. After drying for 5 min, the tabs were examined via front surface room-temperature fluorescence spectroscopy to reach limits of detection (LODs) at the parts-per-billion ($ng \cdot mL^{-1}$) level [5,13]. Later reports extended the use of SPE membranes to the room temperature phosphorimetry (RTP) analysis of PAHs [6,14–17], polychlorinated biphenyls [15,17] and polychlorinated dibenzofurans [17]. Bulky glassware and vacuum pumps, common to classic SPE lab procedures, were replaced with a syringe kit well-suited for manual extraction under field conditions. A rapid air-drying step, which was manually accomplished by applying positive pressure to the syringe, removed the excess of water from the extraction membrane prior to spectroscopic measurements. Total analysis time took less than 10 min per sample and provided LODs at the parts-per-billion ($pg \cdot mL^{-1}$) concentration level.

This article focuses on the urine analysis of PAH metabolites via SPE-RTF. Although chromatographic techniques provide reliable results for the analysis of PAH metabolites [18–28], the development of easy-to-use and cost effective techniques with high sample throughput is relevant for the assessment of PAHs uptake by large populations [22]. Previous articles from our group targeted monohydroxy-PAHs (OH-PAHs) [29,30]. Signal reproducibility from measurements on extraction membranes was improved with the aid of a sample holder specifically

* Corresponding author.
E-mail address: andres.campiglia@ucf.edu (A.D. Campiglia).

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designed for the manual optimization of luminescence signals. Background correction of solid substrates was carried out with the aid of Asymmetric Least Squares (ALS), a smoothing algorithm originally devised for baseline correction of chromatographic data [31–33]. 2-hydroxy-fluorene (2OH-FLU), 1-hydroxypyrene (1OH-PYR), 3-hydroxy-benzo[*a*]pyrene (3OH-B[*a*]P) and 9-hydroxy-phenanthrene (9OH-PHE) were determined at the parts-per-trillion concentration level with relative standard deviations (RSDs) varying from 3.5% (2OH-FLU) to 9.5% (9OH-PHE). The application of ALS to SPE-RTF improved the LODs by approximately two orders of magnitude. With only 10 mL of urine sample, the LODs of OH-PAHs varied from 57 pg mL^{-1} (2OH-FLU) to 2 pg mL^{-1}(1OH-PYR). Recovery values from urine samples varied from 99.0 ± 1.2% (3OH-B[*a*]P) to 99.9 ± 0.05% (1OH-PYR) [29].

Herein, the application of SPE-RTF is extended to the urine analysis of benzo[*a*]pyrene-*trans*-9,10-dihydrodiol (B[*a*]P-diol), benzo[*a*]pyrene-r-7,t-8,c-9-tetrahydrotriol (B[*a*]*P*-triol) and benzo[*a*]pyrene-r-7,t-8,c-9,c-10-tetrahydrotetrol (B[*a*]P-tetrol). RSDs varying from 2.1% (B[*a*]P-triol) to 8.6% (3-OH B[*a*]P) were obtained with the aid of a fiber optic probe that eliminates the need for manual optimization of signal intensities. ALS background correction provided LODs at the pg·mL^{-1} range. Analytical recoveries from human urine samples varied from 87.6 ± 3.1% (3OH-B[*a*]P) to 99.8 ± 2.5% (B[*a*]P-tetrol). The excellent analytical figures of merit and the simplicity of the experimental procedure demonstrate the potential of SPE-RTF for screening biomarkers of PAH exposure in numerous urine samples.

2. Experimental

2.1. Chemicals and materials

All solvents were Aldrich HPLC grade. All chemicals were analytical-reagent grade and utilized without further purification. Unless otherwise noted, Nanopure water was used throughout. B[*a*]P-diol, B[*a*]P-triol and B[*a*]P-tetrol were purchased from Sigma–Aldrich. 3OH-B[*a*]P was from Midwest Research Institute. All other chemicals were purchased from Fisher Chemical. The Sep-Pak C-18 membranes were purchased from Varian/Agilent. The synthetic urine solution was manufactured by RICCA Chemical Company (Arlington, TX) and purchased from Fischer Scientific. Human urine samples obtained from an anonymous volunteer group of healthy non-smoking individuals were pooled, frozen and stored at 4 °C until further analysis.

2.2. Preparation of stock solution of B[a]P metabolites

Stock solutions of B[*a*]P metabolites (100 μg/mL) were prepared by dissolving 1.0 mg of standards in 10 mL of methanol. All stock solutions were kept in the dark at 4 °C. Prior to use, stock solutions were monitored via RTF spectroscopy for possible photo-degradation of metabolites. All stock solutions were used within 6 months of preparation. Working solutions of B[*a*]P metabolites were prepared daily by serial dilution of stock solutions.

2.3. Hydrolysis of urine samples

Hydrolysis of urine samples followed the procedure reported previously [29,30]. 8 mL of urine sample were spiked with 1 mL of metabolite stock solution of appropriate concentration and equilibrated for 30 min to allow for the interaction of B[*a*]P metabolites with urine components. Then 500 μL of 0.1 M HCl were added to the sample and the mixture was buffered with 500 μL of 0.05 M potassium biphthalate sodium hydroxide buffer (pH 5.0). The buffered sample was shaken for 30 min at 1400 rpm to allow for urine hydrolysis.

2.4. SPE with octadecyl membranes

A cork borer with an inside diameter of 8 mm was used to dissect 47 mm C-18 membranes into 8 mm extraction disks. 8 mm disks were individually loaded into a stainless steel filter syringe kit (Alltech) and then connected to a 10 mL Hamilton syringe. Manual positive pressure forced all liquid solutions through the disk. The optimization of experimental parameters for best retention of B[*a*]P metabolites on extraction membranes led to the following procedure: standard metabolite solutions and urine samples were processed through extraction membranes previously conditioned with 5 mL methanol and 5 mL water. Following sample extraction, each membrane was sequentially rinsed with 5 mL of water and 5 mL of 1% ammonium hydroxide. Void water was mechanically removed from the membrane by 300 mL of air through the disk with the 100 mL syringe.

2.5. Fluorescence background treatment of extraction membranes

Reduction of fluorescence background from extraction membranes was accomplished with a thin layer chromatography (TLC) procedure previously developed in our lab [29]. Each chromatographic run was carried out with 34 mm × 40 mm membranes strips immersed 5 mm deep in methanol for approximately 15 min. This time period was enough for the fluorescence background to reach the maximum migration distance towards the top of the strip. Each membrane strip was submitted to 3 chromatographic runs to achieve the minimum fluorescence background possible. The extraction disks used for SPE-RTF measurements were cut from the strip areas with low fluorescence backgrounds.

2.6. RTF measurements

Fig. 1 shows a schematic diagram of the fiber optic probe (FOP) and the instrumentation used in these studies. The FOP consisted of one delivery and six collection fibers. All the fibers were 3 m long and 500 μm-core diameter silica-clad silica with polyimide buffer coating (Polymicro Technologies, Inc.). The fibers were fed into a 1.2-m-long section of copper tubing that provided mechanical support for lowering the probe into the liquid helium. At the sample end, the fibers were arranged in a conventional six-around-one configuration with the delivery fiber in the center, bundled with vacuum epoxy (Torr-Seal, Varian Vacuum Products), and fed into a metal sleeve for mechanical support. The copper tubing was flared stopping a Swage nut tapped to allow for the threading of a 0.75-mL polypropylene sample vial. At the measurement end, the collection fibers were bundled with vacuum epoxy into a slit configuration, fed into a metal sleeve, and aligned with the entrance slit of the spectrometer.

Fluorescence measurements were carried out with a FluoroMax-3 (Horiba Jobin Yvon, Edison, NJ) equipped with a 450 W xenon arc source. The 1200 grooves/mm gratings in the single excitation and emission monochromators were blazed at 330 and 500 nm, respectively. Their reciprocal linear dispersion was equal to 4.25 nm/mm. The uncooled photomultiplier tube (Hamamatsu, Model R928) detector was operated in the photon- counting mode. Commercial software (DataMax, version 2.20, Horiba Jobin Yvon) was used for automated scanning and fluorescence data acquisition. The excitation fiber and the emission bundle of the fiber optic probe (FOP) were coupled to the sample compartment of the spectrofluorimeter with the aid of a commercial fiber optic mount (F-3000, Horiba Jobin Yvon) that optimized collection efficiency via two concave mirrors. Position alignment of each end of the FOP with the respective focusing mirror was facilitated using commercially available adapters (Horiba Jobin Yvon).