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A simultaneous analysis method of polycyclic aromatic hydrocarbons, nicotine, cotinine and metals in human hair☆

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), nicotine, cotinine, and metals in human hair have been used as important environmental exposure markers. We aimed to develop a simple method to simultaneously analyze these pollutants using a small quantity of hair. The digestion performances of tetramethylammonium hydroxide (TMAH) and sodium hydroxide (NaOH) for human hair were compared. Various solvents or their mixtures including *n*-hexane (HEX), dichloromethane (DCM) and trichloromethane (TCM), HEX:DCM32 (3/2) and HEX:TCM73 (7/3) were adopted to extract organics. The recoveries of metals were determined under an optimal operation of digestion and extraction. Our results showed that TMAH performed well in dissolving human hair and even better than NaOH. Overall, the recoveries for five solutions were acceptable for PAHs, nicotine in the range of 80%–110%. Except for HEX, other four extraction solutions had acceptable extraction efficiency for cotinine from HEX:TCM73 (88 ± 4.1%) to HEX:DCM32 (100 ± 2.8%). HEX:DCM32 was chosen as the optimal solvent in consideration of its extraction efficiency and lower density than water. The recoveries of 12 typical major or trace metals were mainly in the range of 90%–110% and some of them were close to 100%. In conclusion, the simultaneous analysis of PAHs, nicotine, cotinine, and metals was feasible. Our study provided a simple and low-cost technique for environmental epidemiological studies.

1. Introduction

Human exposure to environmental pollutants has been considered as an important factor to disease burden (Briggs, 2003; Hanninen et al., 2014). The potential health effects of organic pollutants and toxic metals have been widely reported (Araujo and Nel, 2009; Li et al., 2006; Lippmann et al., 2003; Wang et al., 2013). Polycyclic aromatic hydrocarbons (PAHs), mainly generated during the incomplete combustion of fossil fuels and biomass (Shen et al., 2010), are a group of important organic pollutants to cause adverse human health outcomes (Nielsen et al., 1996; Wang et al., 2015). Nicotine and cotinine, as two important markers of active or passive smoking, have been used to assess adverse health effects of smoking (Eskenazi and Bergmann, 1995; Eskenazi and Trupin, 1995). In addition, potential effects of heavy metals on public health have been also attracting wide concerns, especially near mining area (Li et al., 2014). Hence, accurate assessments of human exposure to PAHs, nicotine, cotinine, and metals are pretty important in evaluating the health effects of these pollutants on public health.

Hair sampling, as an non-invasive sampling technique, has been used to indicate human integral exposure to pollutants from endogenous and exogenous sources, including PAHs, nicotine, cotinine, and metals (Bartolome et al., 2014; Carneiro et al., 2011; Dogan and Kaya, 2010; Toriba et al., 2003; Yang et al., 2007; Zhang et al., 2007). In epidemiological studies, we usually cannot analyze all of the mentioned pollutants simultaneously because of the quantity limitation of hair samples, especially when

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researchers only focus on specific sections of hairs. For example, sodium hydroxide (NaOH) was used to digest hair sample to determine the contents of PAHs, nicotine and cotinine in human hair samples (Pichini et al., 1997b; Toriba et al., 2003), while nitric acid (HNO_3) or a mixture of HNO_3 and hydrogen peroxide (H_2O_2) was used to digest hair sample in a microwave system for dissolving metals (Zaitseva et al., 2015). PAHs, as non- or weak polar compounds, were usually extracted by non-polar solvents like *n*-hexane (HEX) or cyclohexane (Brzeznicki et al., 1997; Toriba et al., 2003). In contrast, nicotine and cotinine are polar compounds, which need polar solvents like dichloromethane (DCM) or trichloromethane (TCM) for extraction (Chetiyanukornkul et al., 2004; Yasuda et al., 2013). These mutually exclusive operations of digestion and extraction not only result in a high demand of hair quantity, but also cost long time and much reagents, which can be both saved by simultaneous analysis. It is therefore necessary to develop a technical protocol to digest and extract hair sample for analyzing these pollutants simultaneously in a simple way.

There are three challenges to determine the contents of organic and metal components simultaneously. First, choose a digestion solution to digest the hair adequately without interfering with the analysis of metals. Second, find a solution to keep high extraction performances of both polar and non-polar solvents. Third, the effects of organic extraction operations on metal analysis should be well controlled. NaOH has been widely used to digest hair to analyze nicotine, cotinine and PAHs with the concentrations varying from 0.5 M to 2.5 M (Chetiyanukornkul et al., 2004; Pichini et al., 1997b; Schummer et al., 2009; Toriba et al., 2003; Yang et al., 2007), suggesting that NaOH (2.5 M) had good performance for digesting human hair. However, the abundant Na^+ from NaOH solution will cause severe matrix effect for metal quantification. Therefore, this digestion method cannot be applied for the simultaneous analysis of organic compounds and metals. Tetramethylammonium hydroxide (TMAH), as an alternative organic dissolution procedure, has been used to digest human hair for metal analysis (Carneiro et al., 2011; Dogan and Kaya, 2010; Nunes et al., 2000; Rodrigues et al., 2008). Thus, we propose a technical route including two stages briefly presented in a flowchart (see Fig. 1) in consideration of these aspects. In Stage I, we aimed to determine the optimal digestion solution and the ratio of digestion volume to hair weight. Raw human hair sample was utilized and cut into segments. The digestion performance of organic base (i.e. TMAH) was investigated by microscopic observation and compared with inorganic base (i.e. NaOH). The optimal digestion condition was further determined. In Stage II, we aimed to find an optimal extraction method for the quantifications of PAHs, nicotine, cotinine and metals. Standard human hair was digested using the optimal conditions from Stage I. Various organic solvents were used to test their extraction performances for PAHs, nicotine and cotinine. For the optimal extraction solvent, the metal recovery was tested. Our study aimed to investigate the feasibility of the above experiment design.

2. Materials and method

2.1. Reagent and materials

Dichloromethane and trichloromethane were sourced from J.T. Baker® (USA) and *n*-hexane (Ultra Resi-Analyzed®) from Merck KGaA (Darmstadt, Hesse-Darmstadt, Germany). Tetramethylammonium hydroxide (25% m/v, in H_2O), sodium hydroxide, triton X-100, and nitric acid (68%) were bought from Sigma-Aldrich (USA). Standards of acenaphthene-d10, anthracene-d10, chrysene-d12, and perylene-d12, nicotine, and cotinine, and diphenylamine were purchased from AccuStandard, Inc. (New Haven, CT, USA). Human hair standard [GBW07601(GSH-1a)], which contains certified concentrations of metals, was bought from National Standard Material Center in China (bought from the website: www.gbw114.org/d_8780.htm). All glassware was heated at 450 °C for 6 h.

2.2. Hair digestion and microscopic observation

Raw human hair sample (~25 mg) was cut into segments with the length (3–5 mm) and transferred into a washed 2-mL glass vial for each sample. Hair samples and blank vials were washed by 1 mL Triton X-100 (vortex for 5 min), 1 time; 1 mL deionized water (vortex for 5 min), 3 times; and 1 mL mixture of HEX and DCM (3/2, v/v) (vortex for 5 min), 2 times. Add TMAH or NaOH solution of various volumes (0.1–0.5 mL) into the vial and digest hair sample in the ultrasonic cleaner (KQ-500B, Kunshan, China) for 60 min. Homogenize the digestion mixture by vortex mixing for 1 min. Draw a drop of the mixture on glass slide for microscopic observation by stereoscopic microscope (Model: SZX10, Olympus Co.) to determine the performance of digestion solution.

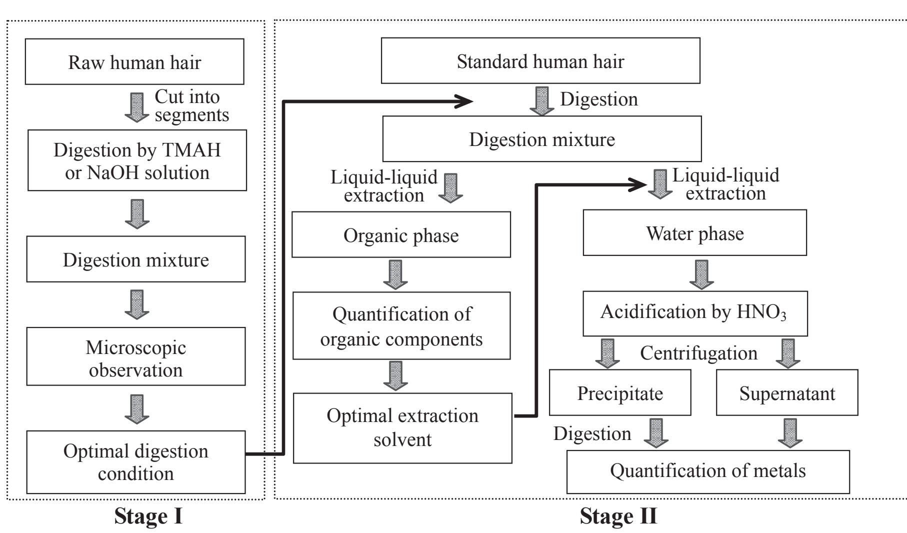

Fig. 1. Flowchart of the extraction and quantification of organic compounds and metals in human hair samples. TMAH, tetramethylammonium hydroxide.