Materials Science and Engineering C 70 (2017) 41–53

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Rapid, efficient and selective preconcentration of benzo[*a*]pyrene (BaP) by molecularly imprinted composite cartridge and HPLC

Mehmet Emin Çorman [a,b,*], Canan Armutcu [a], Lokman Uzun [a,**,1], Adil Denizli [a]

[a] Hacettepe University, Department of Chemistry, Ankara, Turkey
[b] Sinop University, Department of Bioengineering, Sinop, Turkey

ARTICLE INFO

Article history:
Received 3 May 2016
Received in revised form 1 July 2016
Accepted 14 August 2016
Available online 15 August 2016

Keywords:
Polycyclic aromatic hydrocarbons
Benzo[*a*]pyrene
Composite cartridge hydrophobic cryogel
Molecular imprinting
Preconcentration

ABSTRACT

In this study, cryogel-based molecularly imprinted composite cartridges were designed for the rapid, efficient, and selective preconcentration of benzo[*a*]pyrene (BaP) from water samples. First, a BaP-imprinted poly(2-hydroxyethyl methacrylate-N-methacryloyl-(L)-phenylalanine) composite cartridge was synthesized under semi-frozen conditions and characterized by scanning electron microscopy, elemental analysis, Fourier transform infrared spectroscopy, and swelling tests. After the optimization of preconcentration parameters, i.e., pH and initial BaP concentration, the selectivity and preconcentration efficiency, and reusability of these cartridges were also evaluated. In selectivity experiments, BaP imprinted composite cartridge exhibited binding capacities 3.09, 9.52, 8.87, and 8.77-fold higher than that of the non-imprinted composite cartridge in the presence of competitors, such as benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), indeno[1,2,3-*cd*]pyrene (IcdP), and 1-naphthol, respectively. The method detection limit (MDL), relative standard deviation (RSD) and preconcentration efficiency (PE) of the synthesized composite cartridge were calculated as 24.86 μg/L, 1.60%, and 349.6%, respectively.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), produced by the incomplete combustion of organic matter during natural processes as well as industrial and human activities, are hazardous organic compounds comprising two or more fused aromatic rings [1,2]. Because of their high abundance and broad distribution in the environment caused by abundant sources, PAHs are released in air, soil, food, as well as drinking water [3], and humans are exposed to them as part of everyday living. They are considered to be most toxic analytes because some of them exhibit mutagenic and carcinogenic properties, particularly, benzo[*a*]pyrene (BaP) [4]. Recently, BaPs have attracted significantly more attention as they exhibit severe toxic effects even at low concentrations [5]. After BaP is absorbed in the body, it is metabolized to reactive intermediates, such as BaP diol epoxides, which are ultimate carcinogenic metabolites and act as a procarcinogen. These molecules strongly bind to DNA via covalent bonding; as a result, the modification of DNA is responsible for the disruption of DNA and gene transcription, resulting in mutations, tumors, and cancer [6]. From previous studies, there is sufficient evidence to confirm that BaP causes cancer in humans, such as lung, liver, and skin [7]. Because of the harmful effects exhibited by BaP, it has been recognized as a marker for the detection of carcinogenic PAHs [8]. Hence, PAH measurements play a critical role in environmental analysis, which tends to require different separation processes. Various separation and quantification techniques, such as high-performance liquid chromatography (HPLC) equipped with an ultraviolet (UV-DAD) or fluorescence detector (FLD) and gas chromatography with mass spectrometry (GC–MS), have also been proposed for the final determination of PAH in environmental matrices [9–11]. These techniques have been successfully employed and developed for the identification and determination of PAHs. Nevertheless, before chromatographic detection, preconcentration and pre-separation extraction techniques, such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE) procedures, must be applied for separating PAH from aqueous media [12]. These techniques are a suitable technology for pretreatment systems; however, increased flow resistance, pore diffusion, and non-specific sorption hinder their applications.

For addressing these drawbacks, different separation techniques, such as the use of cryogel-based materials, have been suggested as possible alternatives [13]. For this purpose, we focused our efforts on the combination of molecular imprinting techniques with cryogel-based adsorbents for developing new-generation hybrid materials. The molecular imprinting technique is a novel method for creating artificial

* Correspondence to: M.E. Çorman, Sinop University, Department of Bioengineering, 57000 Sinop, Turkey.
** Corresponding author.
E-mail addresses: mecorman@sinop.edu.tr (M.E. Çorman), lokman@hacettepe.edu.tr, lokman.uzun@liu.se (L. Uzun).
[1] Current address: Linköping University, Department of Physics, Chemistry and Biology (IFM), Biosensors and Bioelectronics Division, SE-581 83, Linköping, Sweden.

http://dx.doi.org/10.1016/j.msec.2016.08.040
0928-4931/

antibody-like solid matrices for a wide variety of target molecules. Molecularly imprinted polymers (MIPs) have been investigated for the separation and purification of different compounds for selectively separating single-interest analytes from complex analyte samples in several practical applications [14–19]. It can be simplified as it is a unique method for creating polymeric networks with specific recognition abilities and binding sites, which are complementary to the template with the memory of the shape, size and functional groups [20]. Due to the unique properties, such as high selectivity, reusability, ease and low cost of preparation, MIPs are used as solid-phase extraction sorbents for concentrating and selectively separating the analytes from different samples [21].

Besides the advantages of MIPs, cryogel-based adsorbents, which defined as a continuous bed with interconnected supermacropores, have appeared as new materials for solid-phase extraction. These adsorbents exhibit attractive structural features, and making them promising alternatives with valuable potential uses applications in bioseparation and biotechnology [22]. Due to their advantages including high and controlled large interconnected pores, a desired permeability and ideal diffusion dynamics could easily be achieved. Also, the large interconnected pores increase the linear flow rate and reduce the back pressure while improving separation kinetics [23]. Furthermore, particle embedding and particle crosslinking have been investigated for increasing the specific surface area and enhancing hydrophobic interactions [24,25]. In contrast to plain cryogels, composite cryogels (cartridges) developed by particle embedding facilitate the introduction of significantly more binding sites on the surface and near inside of polymeric walls, and so resulting in an improved high adsorption capacity [26].

In this study, a series of different imprinted composite cartridges were successfully developed for selectively separating the template BaP as an alternative solid-phase extraction sorbent. BaP molecules were selectively preconcentrated from aqueous solutions with high performance due to the excellent structural and chemical features of composite cartridges. As mentioned before, the unique structural features of cryogels-based adsorbents were combined with molecular imprinting for introducing artificial recognition ability into the stable polymeric structure for the selective, efficient, rapid, and cost-effective extraction of BaP molecules. The influencing experimental conditions, such as pH, initial BaP concentration, salt, and temperature, were optimized before the preconcentration of BaP from aqueous solutions in a single run. Analytical measurements were conducted on an HPLC equipped with a fluorescence detector (HPLC-FLD) using a Pinnacle PAH column (Pinnacle II PAH). Also, the selectivity experiments were conducted using benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), indeno[1,2,3-cd]pyrene (IcdP) and 1-naphthol as potential competitors for demonstrating the selectivity of the proposed cartridges against BaP molecules. In addition, reusability experiments, as well as adsorption–desorption-regeneration cycles were also conducted for assessing the economic feasibility of the proposed cartridges. The results indicated that cryogels combined with molecular imprinting could be widely applied to improve the preconcentration cartridges for detecting trace amounts of BaP molecules.

2. Materials and methods

2.1. Chemicals and reagents

PAH standards, classified as human carcinogens by Environmental Protection Agency (EPA) such as BaP, BbF, BkF, IcdP, and 1-naphthol, were purchased from Sigma-Aldrich (St. Louis, USA). Monomers, such as 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), L-phenylalanine hydrochloride, methacryloyl chloride, *N*,*N*′-methylene-bis(acrylamide) (MBAAm), ammonium persulfate (APS), *N*,*N*′-azobisisobutyronitrile (AIBN), and *N*,*N*,*N*′,*N*′-tetramethylene diamine (TEMED) were also purchased from Sigma-Aldrich (St. Louis, USA). Acetonitrile (HPLC grade), potassium bromide, sodium chloride, dichloromethane, triethylamine, and sodium hydroxide were obtained from Merck (Darmstadt, Germany). All solvents and chemicals were of analytical reagent grade unless otherwise mentioned. All glassware were placed in a 5% (v/v) nitric acid solution overnight and then rinsed with deionized (DI) water, followed by drying in a dust-free environment before use. All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LPW reverse osmosis unit. N-methacryloyl-(L)-phenylalanine (MAPA) was chosen as hydrophobic functional monomer, which can selectively interact with PAH molecules. The functional monomer was synthesized according to a previous study [27].

2.2. BaP-imprinted polymers

BaP-imprinted polymers were synthesized by in situ bulk polymerization, which is the most widely used method for preparing MIPs [28]. Bulk polymerization was achieved as follows: first, monomer solutions were prepared by dissolving a mixture containing HEMA (1.0 mL), EGDMA (0.5 mL), and pre-polymerization BaP–MAPA complex (100 μmol:200 μmol, 0.5 mL). Second, toluene as a porogenic solvent was added to the monomer solutions. After the monomer mixture was ultrasonicated, AIBN was added into the solutions as an initiator. Next, the mixture was stirred at room temperature while purging nitrogen gas for 15 min. Two-step polymerization was performed at 55 °C for 3 h and then at 75 °C for 3 h in a temperature-controlled water bath. The bulk polymer was crushed into fine particles by using a mortar and subsequently ground and sieved to an appropriate size ranging from 64 to 71 μm for further use. Furthermore, the same method was followed for synthesizing non-imprinted polymers (NIPs) and blank polymers (BLANK) without the addition of template molecule and functional monomer (MAPA), respectively.

2.3. Preparation of composite cartridges

The BaP-imprinted composite cartridge (BaP-MIP) was synthesized by free radical polymerization as follows: Firstly, MBAAm (0.283 g) and HEMA (1.30 mL) as the crosslinker and basic monomer, respectively, were dissolved in 13.7 mL of DI water. Secondly, crushed BaP imprinted fine particles (ranging from 64 to 71 μm) were added to the solution and vigorously stirred for 15 min. Thirdly, after cooling the polymerization mixture in an ice bath for 15 min, APS (20 mg) and TEMED (25 μL) as the initiator and activator pair, respectively, were added to the polymerization mixture. Next, the mixture was immediately poured into syringes (2.5 mL), and polymerization was conducted at −12 °C for 24 h. Subsequently, the composite cartridges were thawed at room temperature, and the unreacted monomers, as well as other residuals, were removed by washing using DI water. Desorption using acetonitrile (ACN:H_2O; 80:20, v/v) was repeated until BaP could not be detected by spectrofluorometry (λ_{ex}: 290 nm, λ_{em}: 430 nm). Then, the composite cartridge was stored at 4 °C in a solution containing 0.01% sodium azide for preventing microbial contamination. Non-imprinted (NIP) and blank (BLANK) composite cartridges were prepared by the same process, except changing the types of fine particles embedded (Table 1).

2.4. Characterization

Fourier transform infrared (FTIR) spectra of the composite cartridges were recorded on an FTIR spectrophotometer (Spectrum One™, Perkin Elmer, Massachusetts, USA). Firstly, the composite cartridges were dried in a vacuum oven for 24 h before analysis. Next, the samples (2 mg) were treated with KBr (98 mg) for forming pellets, followed by recording the spectrum in the wavenumber range of 4000–650 cm^{-1}.

For determining the amount of MAPA incorporated in the hydrophobic composite cartridges, the dried samples were subjected to elemental analysis using an elemental analyzer (Thermo Scientific FLASH 2000