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Dissolution of biological samples in deep eutectic solvents: An approach for extraction of polycyclic aromatic hydrocarbons followed by liquid chromatography-fluorescence detection

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ABSTRACT

A novel sample preparation method based on the complete dissolution of marine biological samples in choline chloride-oxalic acid (ChCl-Ox) deep eutectic solvent was developed for fast and efficient extraction of eight polycyclic aromatic hydrocarbons (PAHs) using minimum volumes of cyclohexane. The extracted PAHs were purified and then measured by high-performance liquid chromatography-fluorescence detection (HPLC-FL). The effect of key parameters on extraction recoveries and precision was investigated. At optimized conditions, the studied samples were dissolved under atmospheric pressure in ChCl-Ox (1:2) at 55 °C for 30 min, which is considerably lower than the temperature used in the classical and current methods. After dissolution, it took approximately 20 min to quantitatively extract the PAHs from ChCl-Ox using 5 mL cyclohexane. Depending on the analyte, the developed method was linear over the calibration range 1.0–250, 2.0–250, and 5.0–250 ng g^{-1}, with $r^2 > 0.996$. The detection limits of the method were between 0.50 and 3.08 ng g^{-1}. The intra-day and inter-day precisions (based on the relative standard deviation, $n = 5$) of the spiked PAHs at a concentration level of 50 ng g^{-1} were better than 12.6% and 13.3%, respectively. Individual PAH recoveries from spiked marine fish and macroalgae samples were in the range of 71.6% to 109.6%. For comparison, the spiked samples were also subjected to the Soxhlet extraction method. The simplicity of the procedure, high extraction efficiency, short analysis time, and use of safe and inexpensive components suggest the proposed method has a high potential for utilization in routine trace PAH analysis in biological samples.

1. Introduction

The increasing social demand for analytical methods and the need for fast, accurate, precise, selective, and sensitive methodologies oblige us to consider the use of reagents that are innocuous, or at least less toxic than those currently used. We should also consider a drastic reduction in the amount of samples, reagents, and solvents employed for analysis, as well as to minimize, decontaminate, and neutralize the waste generated [1].

In nearly all analytical methods used for determining trace organic pollutants, sample preparation, especially involving extraction, occupies a strategic place [2]. This step generally extracts the target analytes from their matrices into solution and renders them suitable for analysis [1,2].

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds that are included in the European Union (EU) and the US Environmental Protection Agency (US EPA) priority pollutant list because of their mutagenic and carcinogenic properties [3–6]. Most people are exposed to PAHs predominantly from dietary sources, especially seafood [7–9]. In the marine environment, PAHs are available biologically to marine species via the food chain, as waterborne compounds, and from contaminated sediments. As lipophilic compounds, they can easily cross lipid membranes and have the potential to accumulate in aquatic organisms, and may interfere in normal DNA functioning [3,10].

The mode of extraction for PAHs is highly dependent on the matrix. For solid-based matrices such as food samples, sediments, soil, and marine organisms, classical extraction methods such as Soxhlet extraction with nonpolar solvents are used [11]. This method utilizes large amounts of solvent as well as sample and is considerably time consuming [12]. Another separation protocol of PAHs from lipophilic products is based on saponification in basic alcoholic solution, a process that requires 2–4 h. Moreover, the presence of alcohol in the hydrolytic mixture interferes with

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the alkylated PAH derivatives [10]. Consequently, current methods tend to minimize the consumption of solvents, sample amount, and extraction time with the aid of additional energy and/or pressure to the mixture of sample and solvent [13]. These methods, namely, microwave-assisted extraction (MAE) [14], supercritical fluid extraction (SFE) [15], pressurized liquid extraction (PLE) [16], and ultrasound-assisted extraction (USE) [17], differ in the way that the energy is supplied to the system and the kind of extracting fluid employed.

Recently, some methods have used microwaves in combination with water instead of organic solvents for extracting PAHs from solid matrices [18]. Although this technique can considerably increase the environmental-friendliness of the method, some target analytes, e.g., 5-ring PAHs, are fairly insoluble in water, leading to a very small amount extracted into the final organic solvent. Therefore, these analytes produce low signals that may just be detectable with highly sensitive instruments such as gas chromatography-mass spectrometry (GC-MS).

An alternate approach for the extraction of analytes from biological samples is the use of ionic liquids (ILs). For example, Germán-Hernández and co-workers utilized aggregates of 1-hexadecyl-3-butyl imidazolium bromide in a focused-microwave-assisted extraction method followed by high-performance liquid chromatography with ultraviolet and fluorescence detection to determine the 15 + 1 EU priority PAHs in toasted cereals [19].

The widespread use of ILs in both academia and industry is attributed to their unique combination of properties that include negligible vapor pressure, high thermal stability, low/no volatility, and ease of handling [20]. But typically, one cannot simply evaporate ILs to recover analytes, as analytical separations are generally performed with organic solvents. Many reports have also highlighted the hazardous nature and poor biodegradability of most ILs [21]. In addition, processes for the synthesis of ILs are not always environmentally friendly because they generally require large amount of salts and solvents for complete anion exchange [22].

To overcome the limitations of high price and toxicity of ILs, a new generation of green solvents—deep eutectic solvents (DESs)—have emerged [23]. A DES is generally composed of two or three non-toxic components that are capable of associating with each other through hydrogen bonds [22]. DESs typically have a very high depression in freezing point and are liquids at temperatures ranging from 21 °C to 70 °C [23]. Choline chloride (ChCl), an inexpensive, biodegradable, and non-toxic quaternary ammonium salt, is widely used as one of the components in the formation of DESs [24]. When combined with non-toxic hydrogen bond donors (HBDs), such as carboxylic acids (e.g., oxalic acid), urea, or polyols (e.g., glycerol), ChCl is capable of rapidly forming a liquid [22–24]. These mixtures can be prepared with high purity and do not react with water, which allows for easy storage. In addition, they are biodegradable, biocompatible, non-toxic, non-flammable, and inexpensive [22]. Charge delocalization that is achieved through hydrogen bonding between the halide anion (e.g., Cl^- in ChCl) with an HBD moiety is responsible for formation of the liquid [23].

Liquid ChCl mixtures have been used in applications in various fields, including drug solubilization [25], biodiesel purification [26], and electrodeposition of metals [23]. Moreover, they have been proven to dissolve macromolecules. For example, in 2013, Dai et al. [27] utilized different types of DESs composed of natural constituents, e.g., proline-malic acid (PMA), for the extraction of phenolic metabolites of various polarities from safflower. Most major phenolic compounds were recovered from the DES with yields between 75% and 97%. They also found that the H-bonding interactions between DES molecules and phenolic compounds are responsible for their high extractability.

Similarly, Bi and co-workers used alcohol-based DESs that are prepared by mixing ChCl with different alcohol-based HBDs to extract flavonoids (myricetin and amentoflavone) from plants [28].

We recently reported the application of some DESs for complete dissolution of marine biological samples, which facilitated the quantitative extraction of some studied metals with small volumes of dilute nitric acid for determination by flame atomic absorption spectrometry (FAAS) [29]. Therefore, based on these experiments, we developed a new, green, and efficient method for the extraction of PAHs from marine biological samples, e.g., fish and macroalgae samples, after their complete dissolution in the studied DESs. The extracted PAHs were then analyzed with high-performance liquid chromatography coupled with a fluorescence detector. Generally, the chemical characteristics of PAHs are similar within a given ring number [30]; therefore, PAHs that contain three, four, and five aromatic rings were selected, because more than 80% of USEPA PAHs belong to these ring classes. As a model, 8 PAHs including phenanthrene (Ph), anthracene (An), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chry), benzo[*e*]pyrene (BeP), and benzo[*a*]pyrene (BaP) were studied. The optimum analytical conditions for quantitative recoveries of PAHs were investigated. To our knowledge, this is the first report of using DESs for the extraction of organic pollutants from solid-based biological matrices.

2. Experimental

2.1. Reagents and solutions

All reagents used in the experiment were of analytical reagent grade and used without further purification. Choline chloride ($C_5H_{14}NClO$, 99.0%) was purchased from Sigma (St. Louis, MO, USA). High-purity oxalic acid (Ox) was supplied by Merck (Darmstadt, Germany). The standard mixture of PAHs (Ph, An, Flt, Pyr, BaA, Chry, BeP, and BaP) containing 500 μg mL^{-1} of each component in toluene was purchased from Supelco (Bellefonte, PA, USA). Stock solutions containing 10 μg mL^{-1} of PAHs were prepared by dilution of the standard mixture in acetonitrile and stored at 4 °C in darkness. Fresh calibration solutions were prepared daily from stock solutions. HPLC-grade acetonitrile, dichloromethane, *n*-hexane, toluene, cyclohexane, and water were purchased from Merck. Double distilled deionized water was used throughout. All glassware was washed three times with *n*-hexane and methanol before use.

2.2. Instrumentation

PAHs were quantitatively determined with the Knauer HPLC instrument (Berlin, Germany) with a Wellchrom series solvent organizer (k-1500), binary pump (k-1001), vacuum degasser, electrical injection system, and RF-10 AXL fluorescence detector. The column temperature was maintained at 25 °C in an oven (S-4000) with a thermostat. PAHs were separated on a Nucleosil® LC-PAH column (250 mm × 4.6 mm i.d., particle size 5 μm, pore size 100 Å) from Macherey-Nagel (Düren, Germany). A personal computer equipped with Ezchrom 3.1.7 software (Knauer) for the LC system was used to acquire and store the chromatographic data. A rotary evaporator (Heidolph Laborota 4011-digital, Germany) with bath temperature ≤30 °C was used for the fast solvent evaporation of the extracts.

2.3. Sample collection and pretreatment

In this study, two types of marine fish samples i.e., Kafshak (*Platichthys flesus*) and Shoorideh (*Otolithes ruber*) were bought fresh from a local fish market in Khorramshahr, Iran. These fish