Hydrophobic deep eutectic solvents as "green" extraction media for polycyclic aromatic hydrocarbons in aqueous samples

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Abstract: The paper presents novel nonionic and hydrophobic deep eutectic solvents which were synthesized from natural compounds, *i.e.,* thymol, ±camphor, decanoic and 10-undecylenic acids. Fundamental physicochemical properties of the synthesized deep eutectic solvents were determined, followed by their application as extractants in ultrasound-assisted dispersive liquid-liquid microextraction to isolate and enrich polycyclic aromatic hydrocarbons from aqueous samples characterized by a complex matrix. The final determination was carried out by gas chromatography-mass spectrometry. The most important extraction parameters were optimized and the procedure was validated. The developed procedure is characterized by low limits of detection and quantitation, equal to 0.0039 – 0.0098 µg/L and 0.012 – 0.029 µg/L, respectively, good precision (RSD < 6.09%), analyte recovery ranging from 73.5 to 126.2%, and a wide linear range. The procedure was applied to analysis of industrial effluents from the production of bitumens before and after treatment by advanced oxidation processes. A total of 16 PAHs at concentrations ranging from 0.12 to 46.2 µg/L were identified and determined.

Keywords: deep eutectic solvents, dispersive liquid-liquid microextraction, sample preparation, gas chromatography, water analysis, polycyclic aromatic hydrocarbons.

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1. Introduction

The term polycyclic aromatic hydrocarbons (PAHs) is used to describe a group of organic compounds consisting of two or more fused aromatic rings and not containing heteroatoms or any substituents. According to the US EPA, 16 PAHs were classified in 1976 as priority environmental pollutants due to their carcinogenic and mutagenic properties and the availability of standards and instrumentation enabling their determination at low concentration levels. It has now been established that also other PAHs, not included in the priority 16 PAHs, play an important role in environmental pollution [[1]-[4]]. PAHs are formed mostly as a result of activities of various industries, including oil refinery and petrochemical [[5]-[7]], textile [[8]], paper [[9]] or fossil fuels processing [[10]], resulting in generation of large volumes of effluents rich in these compounds. The main problem in determination and identification of PAHs in samples of industrial effluents is their low concentration and a very complex matrix. Consequently, their analysis requires a sensitive and selective method of determination, such as gas chromatography (GC) [[7],[11]-[17]] or liquid chromatography (LC) [[18]]. Isolation and enrichment of analytes is required prior to their determination by GC or LC.

Over the last few years, a number of classic extraction techniques have been replaced by so-called green extraction techniques [[19]]. The most important feature of green extraction techniques is a complete elimination of volatile, toxicand hazardousorganic substancesor replacing them with less toxic solvents. Some of the extraction techniques available, such as static headspace (SHS), dynamic headspace (DHS) or solid phase microextraction (SPME) do not require the use of solvents; however, their application is limited by insufficient sensitivity toward high-boiling point components, such as PAHs, a long time of analysis or the need for expensive instrumentation. Therefore, sample preparation often involves liquid-liquid extraction, including dispersive liquid-liquid microextraction (DLLME) [[7]] or single drop microextraction (SDME) [[16]], which have more general applicability and the search for novel, alternative and nontoxic solvents has become an important area of current research [[20]].

Until recently, the research has been focused on ionic liquids (ILs) as an extraction medium due to their unique properties, such as density, viscosity, hydrophilicity or hydrophobicity and solubility, which could be adjusted by the selection of an appropriate cation and anion [[21]-[22]]. However, the problems with their biodegradability, toxicity, stability and an often expensive synthesis make them less than perfect green solvents [[23]]. An alternative to classical ILs are deep eutectic solvents (DES), which have similar properties, but their synthesis is simpler and less expensive and they are more biodegradable and often less toxic than ILs. According to the definition, DESs are formed as a result of specific interactions, mostly hydrogen bonding, between two compounds, one of which is a hydrogen bond donor (HBD) and the other one is a hydrogen bond acceptor (HBA). The eutectic mixture obtained is characterized by a much lower melting point than either of the two components [[24]-[26]]. The majority of deep eutectic solvents are hydrophilic which precludes their use as extracting agents for aqueous samples. DESs are a new class of green solvents and the research for finding new hydrophobic ones isongoing. So far, only a few papers have been dedicated to hydrophobic DESs, which can be obtained by combining choline chloride with phenolic compounds [[27]-[29]], menthol or quaternary ammonium salts with carboxylic acids [[30]-[33]], or DESs obtained solely from carboxylic acids [[34]].

The paper discusses novel hydrophobic nonionic deep eutectic solvents based on a combination of thymol [Th] with ±camphor [C] and thymol with 10-undecylenic [UA] and decanoic [DA] acids.

Fundamental physiochemical properties of the new deep eutectic solvents are provided along with their use as extractants in ultrasound-assisted dispersive liquid-liquid microextraction (USA-DLLME) to isolate and enrich PAH analytes from industrial effluents. Final determination of the analytes was carried out by gas chromatography-mass spectrometry (GC-MS).

2. Experimental

2.1. Materials

The following reagents were used in this study: thymol (purity ≥ 99.0%) and ±camphor (purity ≥ 95%) were purchased from Sigma-Aldrich (USA), decanoic acid (purity≥ 98%) and 10-undecylenic acid (purity ≥ 97%) were purchased from Merck (Germany). Solvents: methanol (MeOH), acetone (AC), acetonitrile (AcCN), isopropanol (IPA) (purity ≥ 99.9%) were purchased from POCH (Poland). High purity standards: naphthalene, biphenyl, acenaphthylene, acenaphthene, fluorene, fluoranthene, phenanthrene, anthracene, 9-methyl anthracene, pyrene, benz[a]anthracene, chrysene, triphenylene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, benzo[b]chrysene and perylene (purity ≥ 98%)as well as internal standards: 1-chloronaphthalene (purity ≥ 85%), acenaphthene-d10 (purity ≥ 99%) and anthracene-d10 (purity ≥ 98%) were purchased from Sigma-Aldrich (USA).

2.2. Real samples

Effluents from the production of bitumen 20/30 (penetration grade 20-30 and softening point 55-63 °C, used as a paving grade bitumen suitable for road construction and repair) from vacuum bottoms of crude oil Rebco:Kirkuk 65:35 m/m (mixture of Russian and Iraqi crude oils) were collected behind a plate separator which separated condensed organic phase from aqueous phase. The aqueous phase of raw effluents was investigated along with the effluents subjected to various chemical treatments, including hydrodynamic cavitation (HC) aided by peroxone (mixture of O_3/H_2O_2) and HC combined with sodium peroxydisulfate ($Na_2S_2O_8$). A detailed characteristic of the effluents was provided in previous papers [[35]-[39]].

2.3. Apparatus

A model QP2010 GC–MS SE gas chromatograph-mass spectrometer (Shimadzu, Japan) equipped with a combi-PAL AOC 5000 autosampler (Shimadzu, Japan) and an HP-5 ms (60 m × 0.25 mm × 0.25 µm) capillary column (Agilent, USA) were used in the investigations. LabSolutions software (Shimadzu, Japan) with NIST 14 and Wiley 8.0 mass spectra library were used for data management. An RK 156 BH ultrasonic bathwith 35 kHz frequency and 6-L capacity (BANDELIN electronic GmbH& Co. KG, Germany) was used for extraction and an EBA 8S centrifuge (Hettich, Germany) was used for the separation of extracts from samples. FT-IR spectra were obtained using a Bruker Tensor 27 spectrometer (Bruker, USA) with an ATR accessory and OPUS software (Bruker, USA). ^1H NMR and ^{13}C NMR spectra were taken using a Varian Unity Inova 500 MHz spectrometer (Varian, USA). Dynamic viscosity was determined with a BROOKFIELD LVDV-II+ viscometer (Labo-Plus, Poland). Melting points were determined using a CC 906w cryostat (Huber, Germany).

2.4. Procedures

2.4.1. Synthesis of DES

Deep eutectic solvents were synthesized by combining thymolwith ±camphor, 10-undecylenic acid, or decanoic acid, respectively,so as to obtain mixtures with a mole ratio of 7:3, 3:2, 1:1, 1:2, 1:3 and 1:4. Next, the mixtures were stirred magnetically at 60°C until homogeneous liquids were obtained. The liquids were then left to cool spontaneously to room temperature.

2.4.2. Determination of physicochemical properties

FT-IR spectra of deep eutectic solvents and their components were taken using attenuated total reflectance (ATR) with the following operating parameters: spectral range 4000-550 cm^{-1}, resolution: 4 cm^{-1}, number of sample scans: 256, number of background scans: 256, slit width: 0.5 cm.

In order to take ^{1}H NMR and ^{13}C NMR spectra, DES solutions were prepared in 5 mm NMR tubes by weighing 40 mg of a DES and adding 0.5 mL of dimethyl sulfoxide (DMSO). The measurements were carried out at room temperature (RT).

The determination of dynamic viscosity and density was performed at RT and atmospheric pressure. Viscosity was measured using 5 mL of a DES whereas density was determined gravimetrically using 1 mL of a DES. Melting point (MP) was determined visually at atmospheric pressure by cooling DES samples to – 45 °C, followed by a temperature increase at 0.5 °C/min. The initial temperature at which the phase change of DES occurred was taken as the melting point.

2.4.3. Extraction procedure

Effluent samples (10 mL) were transferred to 12-mL vials, followed by an addition of 20 µL of internal standards solutions (1-chloronaphtalene, acenaphthene-d10, anthracene-d10) in AcCN at a concentration of 0.5 mg/mL. Next, NaCl was added (10%, w/v) and a pH adjusted using a 7% HCl solution. A mixture consisting of 200 µL [Th][C] 1:1 and 500 µL of AcCN was then introduced to the sample, the vial was closed and placed in an ultrasonic bath at 25 °C for 14 min. Subsequently, the sample was centrifuged for 5 min at 4000 rpm, and the 180 µL extract obtained was transferred into 2-mL vials with 300 µL micro inserts, using a 100-µL automatic pipette.

2.4.4. Chromatographic analysis

Temperature program: 50 °C (2 min) – ramped at 10 °C/min to 190°C (0 min) – ramped at 5 °C/min to 280 °C (5 min) – ramped at 5 °C/min to 310 °C (1 min); injection port temperature 330 °C; injection volume 2 µL; injection mode: splitless; detector temperature 310 °C; ion source temperature (EI, 70 eV) 220 °C; GC/MS transfer line temperature 300 °C; the carrier gas was hydrogen (1 mL/min).

3. Results and discussion

3.1. Synthesis and physicochemical properties of DESs

If DESs are to be used as extraction solvents, they must be liquid at the extraction temperature. The compounds used to prepare them (Figure 1) in their pure form are all solids at room temperature with melting points for [Th], [C], [UA] and [DA] equal to 49.6, 179.8, 24.5 and 31.5 °C, respectively. Among the investigated DESs, only some were liquids at ambient temperature, including [Th][C] at mole ratios 7:3, 3:2 and 1:1; [Th][UA] over the entire investigated range: 7:3, 3:2, 1:1, 1:2, 1:3, and 1:4 while [Th][DA] was liquid at mole ratios 3:2, 1:1, 1:2 and 1:3 (Figures S1-S3). Specific values of melting points of the investigated deep eutectic solvents are listed in Table 1. Solid-liquid phase diagrams of the DESs are shown in Figure 2. The diagrams reveal a significant depression of DES melting points with respect to the pure components. An especially large depression is observed for the DES formed from [Th] and [C] 1:1, for which the MP is -44.0 °C.

In order to elucidate the structure of the DESs formed, ^{1}H NMR and ^{13}C NMR spectra were taken. Examples of the spectra for samples of [Th][C], [Th][UA] and [Th][DA] at a 1:1 mole ratio are shown in Figure 3 and Figures S4-S5, respectively. All the peaks in the spectra can be assigned to the DES components; no additional peaks which would indicate the occurrence of side reactions are observed.

In addition, in order to elucidate the interactions between the two components resulting in the formation of DESs, FT-IR spectra were recorded. A comparison of FT-IR spectra of pure components and the DESs formed is shown in Figure 4 and Figures S6-S7. The synthesis of deep eutectic solvents is accomplished by the formation of hydrogen bonds between HBA and HBD. The location of the bonds depends on the structure of the reactants. In ionic deep eutectic solvents, for example those formed by the combination of choline chloride with phenols, hydrogen bonds are formed in the vicinity of –O-H groups of phenols, which is indicated by a shift of the O–H band for a pure HBD towards lower wavenumbers [[40]]. An inspection of FT-IR spectra of all the investigated deep eutectic solvents also reveals a shift of the O-H band of [Th] from 3207.7 cm^{-1} [Th] to 3400.2, 3435.7, and 3441.9 cm^{-1} for [Th][C], [Th][UA], and [Th][DA], respectively. In addition, the spectra of DESs formed from thymol and carboxylic acids reveal a characteristic shift of the bands corresponding to stretching vibrations of the carbonyl group towards higher wavenumbers: from 1699.1 cm^{-1} to 1722.6 cm^{-1} and from 1698.2 cm^{-1} to 1725.5 cm^{-1} for [Th][UA] and [Th][DA], respectively, which indicates the formation of new hydrogen bonds in the vicinity of the –COOH group. A similar behavior was also observed in DESs composed of DL-menthol and carboxylic acids [[41]].

The viscosity and density values of the DESs were determined at room temperature. These properties play an important role in mass transfer processes, affecting emulsification and the ease of phase separation in the extraction process. In addition, the average molar masses of the DESs were calculated. All the properties are compiled in Table 1. The viscosity and density values of the DESs for the same mole ratio can be arranged in the following order: [Th][C]>[Th][UA]>[Th][DA].

The synthesis of all the investigated DESs is inexpensive compared to the synthesis of ILs. The cost of 100 g of a DES does not exceed $25 while the price of ionic liquids varies from $250 to $7500. In addition, all DES are characterized by low toxicity due to the fact that they were made of low-toxic natural compounds.

3.2. Optimization of extraction conditions

Optimization of extraction conditions using deep eutectic solvents as the extractants was carried out for several PAHs, including fluorene, phenanthrene, pyrene and benzo[a]pyrene which were selected as representative PAHs. Most of the published research indicate very similar impact of optimized parameters on the extraction efficiency of all PAHs [[11]-[12],[14]-[15]]. The extraction efficiency was estimated from total peak areas of the selected analytes.

3.2.1. Kind of DES

Deep eutectic solvents which were liquid at room temperature were used as extractants.The following conditions were used in studies on selection of the optimum extracting agent: 300 µL DES, 700 µL AcCN, pH 7, 0% w/v NaCl, and 15-min sonication. Among the investigated DESs, the highest extraction efficiency for PAHs was obtained with [Th][C] 1:1. For the DESs with organic acids as one of the components, the extraction yield increased with the [Th] content. This can be attributed to an increase in content of aromatic rings from [Th], which improves the extraction yield of PAHs. Thymol molecules contain the aromatic ring, thus higher concentration of this component in DES will contribute to the increased extent of π-π interactions between the PAHs analytes and the extraction solvent. On the other hand, an opposite phenomenon takes place with [Th][C], wherein an increase in [Th] content lowers the extraction yield. This is likely due to the presence of the polar –OH group in [Th] and the occurrence of a less polar C=O groupin [C], whose increase in content in the DES improves the PAH extraction yield compared to the hydroxyl group.

The effect of kind of DES on the PAH extraction yield is shown in Figure 5.

3.2.2. Kind of disperser solvent

Several disperser solvents were tested in the investigations: AC, MeOH, AcCN and IPA. The following conditions were used: 300 µL [Th][C] 1:1, 700 µL of disperser solvents, pH 7, 0% w/v NaCl, and 15-min sonication. The highest extraction yield of PAHs was obtained with AcCN. The kind of disperser solvent has only a slight effect of extraction yield which is in agreement with a previous work [[7],[42]-[45]]. The effect of disperser solvent on the extraction yield is depicted in Figure 6.

3.2.3. Central Composite Design

The central composite design (CCD), also called the Box-Wilson design [[46]], was selected for the optimization of major parameters potentially affecting the extraction yield. CCD is a five-level factorial design consisting of edge points (+1 and -1) corresponding to the upper and lower limits of the investigated factor, the star points (-α and +α) and the center point. The following extraction parameters were selected: sonication time (2-18 min), salting out effect (0-20% NaCl), DES ([Th][C] 1:1) volume (200-600 µL) and disperser solvent (AcCN) volume (0-1000 µL). The exact values for the five levels arecompiled in Table 2 while the CCD matrix and the results of PAH extraction are listed in Table S1.

The results were analyzed by ANOVA and regression in order to create an appropriate model and select statistically important parameters (Table 3). In ANOVA, the values of statistical parameters F and p were used as criteria at a 95% confidence level. All the parameters for which the p-value was less than 0.05 were considered as statistically significant and having a substantial effect on the model. The developed model was found to be statistically significant due to the p-value < 0.0001 and F-value equal to 50.35 (Table 3). The values of lack of fit (p-values greater than 0.06) were considered insignificant. The response equation (1) obtained for the experimental results is shown below:

$$Y = -28117320 + 2958279 X_1 + 1334994 X_2 + 71672.8 X_3 + 7243.8 X_4 - 93581.4 X_1^2 - 58484.5 X_2^2 - 81.8 X_3^2 - 12.4 X_4^2 - 2063.9 X_1X_3 \quad (1)$$

where Y is the total peak area of the peaks of selected PAHs. The equation explains the effect of the investigated parameters on the PAH extraction yield. The developed model has a large coefficient of determination ($R^2 = 97.92$) along with the values of predicted-R^2 and adjusted-R^2, equal to 89.07 and 95.97%, respectively, which demonstrates a good fit of the model to the experimental values and the possibility of prediction of responses for new data.

3.2.4. Interaction effect

In order to facilitate selection of the optimum values of volumes of DES and AcCN, NaCl concentration and sonication time, the results were plotted as response surfaces (Figure 7). The results demonstrated that the most pronounced effect on the extraction yield have DES volume and sonication time (p-value < 0.002). Figure 7 (A,D,F) reveals that an increase in DES volume from200 µL to 600 µL is accompanied by a significant decrease in the extraction yield. An increase in the extractant volume results in an increase in volume of the extract, thus lowering the concentration of analytes and their peak areas (for the same injection volume). Extending sonication time from 2 to 14 min improved the extraction yield while further extension of time deteriorated the extraction yield (Figure 7 A-C). Extending the time of sonication is particularly advantageous when using small amounts of extraction solvent (Figure 7A). Ultrasounds facilitate dispersion of the extractant thus improving the extraction yield; however, extensively long sonication results in degradation of PAHs in aqueous matrices [[47]]. This is due to acoustic cavitation taking place in aqueous media which can result in degradation of a variety of organic compounds [[48]-[49]].

For the remaining investigated parameters the p-values were greater than 0.273; thus, their effect on the extraction yield was much smaller. A slight improvement in the extraction yield after addition of NaCl at a concentration of 10% w/v, followed by a gradual decrease with further addition of the salt was observed (Figure 7 B,D-E). Theoretically, salt addition should improve the extraction yield by lowering the solubility of organic compounds in the aqueous phase due to an increase in ionic strength; however, an increase in NaCl concentration above 15% w/v increased the density and viscosity of solution thus lowering the effectiveness of emulsification. An improvement in the extraction yield was also observed upon increasing the volume of disperser solvent (AcCN) from 0 µL to 500 µL, followed by a gradual decrease after increasing the volume from 500 µL to 1000 µL. This phenomenon is typical in DLLME, wherein too small a volume of disperser solvent precludes complete emulsification of an extractant, thus lowering analyte recovery. On the other hand, too large a volume of AcCN also decreases the extraction yield, most likely by increasing the affinity of PAHs to the aqueous phase and hence the change in partition coefficient. Theoretically, ultrasonic waves can disperse extraction solvent in sample solution thoroughly, without a disperser solvent. However, these studies revealed that the use of both factors together improves the efficiency of extraction, which can be seen in Figure 7 (C).

Based on the optimization study, the following extraction conditions were used in further experiments: extractant DES [Th][C] 1:1 at 200 µL, disperser solvent AcCN at 500 µL, concentration of

NaCl 10% w/v and sonication time 14 min. Optimization of extraction conditions should also include pH of samples. However, since PAHs do not dissociate in aqueous media, the studies were carried out at a neutral pH (pH 7) [[50]-[51]]. The other optimized extraction parameters are time and speed of centrifugation of samples. These parameters, however, were shown in previous studies to have a minor effect on the extraction yield. Therefore, the values found to be optimal in previous works were used in this study: 5 min centrifugation at 4000 rpm [[7],[43]-[45]].

3.3. Validation of procedure

Quantitative analysis was based on the internal standard method using three internal standards: 1-chloronaphthalene, acenaphthene-d10 and anthracene-d10. Nine-point calibration curves were prepared for concentrations equal to 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 25 and 50 µg/L. Figure S8 presents a chromatogram of a mixture of standards at a concentration of 1 µg/mL. Two characteristic mass-to-charge ratio (m/z) values were determined for each analyte, *i.e.*, target ion, which was used to determine peak areas needed for calibration curves and the determination of concentrations of PAHs in real samples, and the qualifier ion used for confirmation of identification of PAHs present in samples. A compilation of retention times and characteristic ions is shown in Table S2.

The LOD and LOQ values for the majority of PAH analytes ranged from 0.0039 to 0.0098 µg/L and 0.012 to 0.029 µg/L, respectively. Higher LOD and LOQ values were obtained for9-methyl anthracene (0.037 and 0.11µg/L) and for benzo[ghi]perylene (0.021 and 0.063 µg/L). Additionally, a wide linear range and good coefficients of determination (R^2) ranging from 0.9989 to 0.9999 were obtained for all of the analytes. Detailed results are presented in Table 4.

To test the usefulness of the developed procedure, the analyte recovery (R) from samples of deionized water and real effluent was determined by spiking the samples with two concentrations: 1 µg/L and 10 µg/L of the analytes. Satisfactory recoveries were found for the majority of the analytes in both types of samples, ranging from 73.5 to 126.2% and from 79.8 to 103%, respectively. A relatively low recovery was found only for triphenylene (68.3– 72.2%). In addition, the precision of the developed procedure was determined by calculating relative standard deviation (RSD) values from the results of 4 analyses performed on the same day (Intra-day RSD) as well as 6 analyses performed during three consecutive days (Inter-day RSD). The obtained values of RSD, determined at the same concentrations as those for recovery, ranged from 2.14 to 5.61% and from 3.01 to 6.09 % for intra-day RSD and inter day RSD, respectively. All the R and RSD values are compiled in Table 5.

A comparison of the developed procedure with other procedures for the determination of PAHs revealed that the proposed procedure has similar LOD and analyte recovery compared to the existing procedures while offering a significant improvement in precision. In comparison with solid phase extraction (*i.e.,* SPME, SPE) as well as solvent microextraction procedures, *i.e.,* solvent bar microextraction (SBME) or SDME, the developed procedure has a shorter sample preparation time [[12],[14]-[16]]. Furthermore, an advantage of the proposed procedure is the use of a nontoxic extracting solvent, offering an alternative to the most popular procedures using chlorinated solvent to extract PAHs from aqueous samples [[7],[13]-[14]]. A comparison of the new procedure with the one based on Emulsification Liquid–Liquid Microextraction using a Deep Eutectic Solvent (ELLME-DES) revealed that the former ischaracterized by lower LOD values [[53]].

A comparison of the available procedures for the determination of PAHs is provided in Table 6.

3.4. Analysis of real samples

The developed procedure was used for the analysis of samples of effluents from the production of bitumens, both raw and subjected to treatment by hydrodynamic cavitation (HC) aided by peroxone or $Na_2S_2O_8$. The analytes were identified based on the ratio of intensities of characteristic ions listed in Table S2, taking the confidence interval of ± 15 %, and also based on the values of retention times ± 0.1 min.

In samples of raw effluent, 16 PAHs analytes were identified at concentrations ranging from 0.12 to 46.2 µg/L, the most abundant being naphthalene, biphenyl, acenaphthylene, anthracene, benz[a]anthracene and 9-methyl anthracene (Figure 8).In addition, another derivative of 9-methyl anthracene with a substituent in a different position in the ring was tentatively identified in raw effluent as indicated by a peak with a retention time of 25.46 min, which has the same characteristic ions with similar intensities. The results obtained for samples of treated effluent reveal a much better effectiveness of degradation of the method HC/peroxone, which resulted in a complete or almost complete degradation of the majority of the analytes. In contrast, the effluent treated by the HC/$Na_2S_2O_8$ process, PAHs were oxidized to a much lesser extent and the effectiveness of treatment did not exceed 29.85%. Nevertheless, in both cases the effectiveness of degradation of PAHs was higher for the analytes having a lower boiling point.

A compilation of the identified analytes along with their concentrations in raw and treated effluents is provided in Table 7.

4. Summary

The paper presents a novel procedure for the determination of PAHs in aqueous matrices using USA-DLLME-GC-MS. New types of hydrophobic deep eutectic solvents composed of natural, nontoxic compounds, *i.e.,* thymol, ±camphor, decanoic and 10-undecylenic acids were used for the first time as extractants. In contrast with ionic liquids and ionic DESs, the deep eutectic solvent [Th][C] 1:1 selected during the optimization step allows its application in gas chromatographic analyses due to its nonionic nature and volatility of its components. Furthermore, the use of an inexpensive, nontoxic and simple to synthesize DES for the extraction of PAHs from aqueous samples by DLLME is an attractive alternative totoxic chlorinated solvents as it meets all the requirements of green chemistry. The developed procedure is characterized by low LOD and LOQ values, a wide linear range, high analyte recovery and acceptable RSD, thus demonstrating its usefulness for the determination of low concentrations of PAHs in samples having a complex matrix.

The procedure was used in the investigations of effectiveness of treatment of effluents from the production of bitumens, which have a matrix rich in various types of organics. The presence of a number of PAHs at concentrations ranging from 0.12 to 46.2 µg/L in the effluent indicates the need for effective treatment methods [[54]-[55]]. A comparison of two treatment methods based on hydrodynamic cavitation aided by different oxidants, including peroxone and $Na_2S_2O_8$ reveal a much better degradation yield of the method based on the O_3/H_2O_2 mixture.

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Table 1 Compilation of physicochemical properties of DESs.

DES	Mole ratio	X_{Th}	X_2	M_W[g/mol]	ρ [kg/m^3]	H[mPa·s]	MP [°C]
[Th][C]	7 : 3	0.70	0.30	150.82	969.8	18.8	-33.0
	3 :2	0.60	0.40	151.02	973.2	20.5	-37.0
	1 : 1	0.50	0.50	151.23	987.3	25.8	-44.0
[Th][UA]	7 : 3	0.70	0.30	160.44	959.8	15.6	19.0
	3 :2	0.60	0.40	163.84	951.5	14.4	16.5
	1 : 1	0.50	0.50	167.25	945.7	13.2	11.0
	1 : 2	0.33	0.67	172.93	939.5	13.1	10.0
	1 : 3	0.25	0.75	175.77	937.5	12.4	9.0
	1 : 4	0.20	0.80	177.47	935.3	11.8	7.5
[Th][DA]	3 :2	0.60	0.40	159.04	951.5	13.0	18.0
	1 : 1	0.50	0.50	161.24	943.7	11.2	17.0
	1 : 2	0.33	0.67	164.91	936.5	10.8	18.0
	1 : 3	0.25	0.75	166.75	927.2	10.4	19.0

M_W– molar mass, calculated from $M_W=x_{Th}\cdot M_{Th}+x_2\cdot M_2$,where x_1- mole fraction of [Th], M_{Th}– molar mass of [Th] [g/mol], x_2– mole fraction of the second component of DES, M_2–molar mass of the second component [g/mol], MP – melting point [°C].

Table 2 Experimental ranges and levels of two variables in CCD.

Variables	Ranges and levels (star points = $(2^k)^{1/4}$= 2) [1]				
	-α	-1	0	+1	+α
(X_1) Sonication time [min]	2	6	10	14	18
(X_2) Salting out [%,w/v]	0	5	10	15	20
(X_3) Volume of DES [µL]	200	300	400	500	600
(X_4) Volume of AcCN [µL]	0	250	500	750	1000

[1] *k – number of variables = 3*

Table 3 Analysis of variance (ANOVA) for the CCD.

Source of variation	DF 1)	Sum of squares	Mean squares	F-value	p-value
Model	14	2.19E+14	1.57E+13	50.35	<0.0001
X1	1	3.45E+13	5.51E+13	177.00	<0.0001
X2	1	2.18E+11	1.64E+13	52.58	<0.0001
X3	1	6.36E+13	1.53E+13	48.98	<0.0001
X4	1	1.76E+12	1.40E+12	4.69	0.048
X_1^2	1	3.65E+13	6.09E+13	195.46	<0.0001
X_2^2	1	4.07E+13	5.58E+13	179.12	<0.0001
X_3^2	1	1.36E+13	1.81E+13	58.27	<0.0001
X_4^2	1	1.64E+13	1.64E+13	52.74	<0.0001
X_1*X_2	1	4.02E+10	4.02 E+10	0.13	0.724
X_1*X_3	1	1.09E+13	1.09E+13	35.02	<0.0001
X_1*X_4	1	2.91E+11	2.91E+11	0.94	0.349
X_2*X_3	1	3.90E+11	3.91E+11	1.25	0.281
X_2*X_4	1	3.92E+11	3.93E+11	1.26	0.280
X_3*X_4	1	1.11E+11	1.11E+11	0.36	0.559
Residual	15	4.67E+12	3.11E+11		
Lack of fit	10	4.19E+12	4.19E+11	4.34	0.060
Pure error	5	4.83E+11	9.66E+10		
R-Sq = 97.92% R-Sq(pred) = 89.07% R-Sq(adj) = 95.97%					

1) *DF - Degrees of freedom*

Table 4 Analytical characteristics of the developed procedure.

Compound	Calibration curve	R^2	LOD [µg/L]1)	LOQ [µg/L]2)	Linear range [µg/L]3)
naphthalene	y = 1.2827x + 0.0087	0.9998	0.0098	0.029	0.029 – 48.40
biphenyl	y = 1.0725x + 0.0092	0.9992	0.010	0.030	0.030 – 62.83
acenaphthylene	y = 1.3256x + 0.0117	0.9998	0.0082	0.025	0.027 –20.48
acenaphthene	y = 1.2153x + 0.0145	0.9995	0.0091	0.027	0.027 –63.73
fluorene	y = 1.2670x + 0.0231	0.9993	0.0088	0.026	0.026 –49.16
anthracene	y = 1.7512x + 0.0224	0.9989	0.0042	0.013	0.013 –51.20
phenanthrene	y = 2.1778x + 0.0114	0.9997	0.0039	0.012	0.012 –37.23
9-methyl anthracene	y = 0.5900x + 0.0276	0.9989	0.037	0.11	0.11 –52.60
fluoranthene	y = 1.0596x + 0.0253	0.9998	0.0061	0.018	0.018 –51.66
pyrene	y = 1.1109x + 0,0205	0.9996	0.0052	0.016	0.016 –45.26
triphenylene	y = 0.8866x – 0.0034	0.9999	0.0041	0.012	0.012 – 2.66
benz[a]anthracene	y = 1.2401x – 0.0168	0.9996	0.0079	0.024	0.024 – 9.11
chrysene	y = 0.9044x + 0.0051	0.9999	0.0047	0.014	0.014 –1.98
benzo[a]pyrene	y = 0.8657x – 0.0200	0.9999	0.0081	0.024	0.024 -1.22
perylene	y = 1.6716x – 0.0175	0.9991	0.0043	0.013	0.013 –7.88
benzo[b]fluoranthene	y = 0.9112x – 0.0191	0.9997	0.0074	0.022	0.022 –1.09
benzo[k]fluoranthene	y = 0.8944x – 0.0081	0.9991	0.0091	0.027	0.027 –11.06
indeno[1,2,3-cd]pyrene	y = 0.9604x – 0.0165	0.9991	0.0089	0.027	0.027 –10.11
dibenzo[a,h]anthracene	y = 0.8135x – 0.0059	0.9992	0.0094	0.028	0.028 –2.39
benzo[b]chrysene	y = 1.3206x – 0.0056	0.9991	0.0061	0.018	0.018 –2.36
benzo[ghi]perylene	y = 1.5054x – 0.0242	0.9999	0.021	0.063	0.063 –2.45

1) *The limit of detection (LOD) was calculated from: LOD = 3·S/N (S - signal, N – noise),*

2) *The limit of quantitation (LOQ) was calculated from: LOQ = 3·LOD,*

3) *The linearity of calibration curve was estimated using the correlation coefficient (r). In order to confirm an appropriate selection of the linear range, a method based on the determination of a function:* $\frac{y_i/y_{ist}}{x_i/x_{ist}} = f(x_i/x_{ist})$, *where:* y_i*– peak area of analyte [au],* y_{ist} *– peak area of internal standard [au],* x_i *analyte concentration [µg/L],* x_{ist} *– internal standard concentration [µg/L] [52]. The plot y = f(x) also contains the value of average response in the form of a straight line parallel to the abscissa as well as straight lines corresponding to the 95% confidence level. The values fitting into the allowed range of errors indicate linearity of the calibration curve while the values exceeding the confidence interval are considered to be nonlinear.*

Table 5 Recovery of analytes and relative standard deviation of determinations.

Compound	Recovery [%]				Intra-day RSD [%] 3)		Inter-day RSD [%] 4)	
	1 μg/L 1)	10 μg/L 2)	1 μg/L 1)	10 μg/L 2)	1 μg/L	10 μg/L	1 μg/L	10 μg/L
naphthalene	95.3	81.6	96.2	88.3	4.99	4.08	5.04	4.18
biphenyl	88.6	89.3	89.2	94.2	4.58	3.49	4.66	4.24
acenaphthylene	98.3	86.1	105.6	89.0	4.18	3.56	4.20	3.66
acenaphthene	101.1	88.3	102.2	97.8	3.98	3.64	3.98	3.67
fluorene	102.2	83.1	92.7	94.3	2.20	2.14	5.51	3.01
anthracene	126.2	87.9	85.8	79.0	4.90	4.68	4.95	4.72
phenanthrene	114.8	86.6	91.6	87.9	5.02	3.90	5.12	4.11
9-methyl anthracene	108.8	99.2	109.3	103.0	6.02	2.49	6.09	5.50
fluoranthene	90.3	91.5	95.2	99.3	4.54	2.43	4.69	3.23
pyrene	89.6	86.2	91.3	89.8	4.81	3.51	4.99	3.74
triphenylene	68.3	-	72.2	-	4.91	-	4.98	-
benz[a]anthracene	89.6	-	92.4	-	4.29	-	4.44	-
chrysene	108.8	-	112.2	-	3.64	-	3.88	-
benzo[b]fluoranthene	105.8	-	108.1	-	4.86	-	4.95	-
benzo[k]fluoranthene	102.3	-	106.0	-	4.99	-	5.11	-
perylene	92.5	-	95.8	-	4.80	-	4.97	-
benzo[a]pyrene	88.1	84.0	98.2	98.1	4.05	2.95	4.83	3.65
indeno[1,2,3-cd]pyrene	79.6	79.8	113.3	99.8	3.74	3.51	4.81	3.75
dibenzo[a,h]anthracene	73.5	-	110.2	-	2.61	-	4.18	-
benzo[b]chrysene	81.5	-	79.4	-	4.85	-	5.22	-
benzo[ghi]perylene	85.1	-	99.2	-	5.61	-	5.81	-

1) *Recovery (R) determined after addition of analytes to deionized water. (Recovery (R) was calculated from:* $R[\%] = \frac{C_{quant}-C_0}{C_{expect}} \cdot 100\%$, *where:* $C_{quant.}$ *– found analyte concentration in spiked sample [μg/L],* $C_{expect.}$ *– analyte concentration added as spike [μg/L],* C_0 *– found analyte concentration in non-spiked samples [μg/L]).*

2) *Recovery (R) determined after addition of analytes to real effluent.*

3) *Intraday RSD determined after addition of analytes to deionized water and 4 analyses on the same day.*

4) *Interday RSD determined after addition of analytes to deionized water and 6 analyses on 3 consecutive days.*

Table 6 Comparison of the developed procedure with other procedures for the determination of PAHs found in the literature.

Analytical method	Compounds	LOD [μg/L]	RSD [%]	R [%]	LR [μg/L]	Time of extraction	Extraction Solvent	Ref.
SPME-GC-MS	16 PAHs	0.001 – 0.029	<20.0	n.d. 2)	0.01 - 10	45 min	-	[[12]]
LLE-GC-MS	12 PAHs	5 - 15	< 29.5	61 - 120	n.d.	n.d.	DCM	[[13]]
SPE-GC-MS	16 PAHs	0.007 – 0.21	< 9.2	61–116	10 - 1000	~45 min	DCM (for elution of PAHs)	[[14]]
DLLME-GC-MS	12 PAHs	0.36 – 5.1	< 6.45	72 – 102	1 - 200	6 min	DCM	[[7]]
SBME-GC-MS	16 PAHs	0.0002 – 0.0008	< 11.6	71 - 148	0.002 – 0.5	60 min	hexane	[[15]]
SDME-GC-MS	18 PAHs	0.01 – 0.03	< 19.0	36 - 152	0.025 –0.075	30 min	toluene	[[16]]
ELLME-DES-HPLC-UV	7 PAHs	0.02 – 0.7	< 2.0	93 - 103	0.1 - 400	20 min	ChCl:Ph 1:2	[53]
USA-DLLME-GC-MS	21 PAHs	0.0039 – 0.0098	< 6.09	74 – 126	0.01 – 50	< 20 min	[Th][C] 1:1	This method

4) *LR – Linear range, n.d - not described, DCM- dichloromethane, ELLME-DES - Emulsification Liquid–Liquid Microextraction Based On Deep Eutectic Solvent*

Table 7 Concentrations of identified PAHs in samples of effluents before and after treatment.

Compound	**Concentration ± SD [µg/L]**					
	HC/peroxone			**HC/$Na_2S_2O_8$**		
	Raw WW	**WW after treatment**	**Total degradation [%]**	**Raw WW**	**WW after treatment**	**Total degradation [%]**
naphthalene	46.2 ± 2.3	8.03 ± 0.40	82.62	45.1 ± 2.3	35.9 ± 1.8	20.38
biphenyl	26.5 ± 1.11	4.18 ± 0.21	84.23	24.2 ± 1.0	17.16 ± 0.43	29.09
acenaphthylene	13.81 ± 0.69	2.18 ± 0.10	84.21	13.01 ± 0.55	9.78 ± 0.55	24.83
fluorene	0.208 ± 0.0070	<LOD	>>99.99	0.201 ± 0.060	0.141 ± 0.042	29.85
anthracene	7.08 ± 0.35	<LOD	>>99.99	6.69± 0.23	6.27± 0.30	6.28
9-methyl anthracene	5.18 ± 0.26	1.210 ± 0.061	76.64	5.07± 0.18	4.88± 0.21	3.74
fluoranthene	0.213 ± 0.011	<LOD	>>99.99	0.191± 0.009	0.188± 0.008	1.57
pyrene	0.169 ± 0.010	<LOD	>>99.99	0.158± 0.007	0.145± 0.006	8.22
benz[a]anthracene	9.04 ± 0.52	8.12 ± 0.47	10.18	8.08± 0.48	7.98± 0.49	1.24
chrysene	1.441 ± 0.081	1.387 ± 0.078	3.75	1.21± 0.051	1.18± 0.048	2.48
benzo[b]fluoranthene	0.282 ± 0.016	0.267 ± 0.015	5.32	0.265±0.089	0.226± 0.086	6.03
benzo[k]fluoranthene	0.124 ±0.011	0.120 ± 0.010	3.23	0.120± 0.045	0.118 ± 0.013	1.67
perylene	0.281 ± 0.017	0.248 ± 0.015	11.74	0.279± 0.097	0.261± 0.018	6.45
benzo[a]pyrene	0.145 ± 0.091	0.110 ± 0.072	24.14	0.144± 0.050	0.122± 0.011	15.28
indeno[1,2,3-cd]pyrene	0.203 ± 0.012	0.194 ± 0.012	4.43	0.193± 0.010	0.189± 0.010	2.07
benzo[ghi]perylene	0.197 ± 0.011	0.188 ± 0.011	4.57	0.182 ± 0.011	0.172 ± 0.011	5.50

Figures

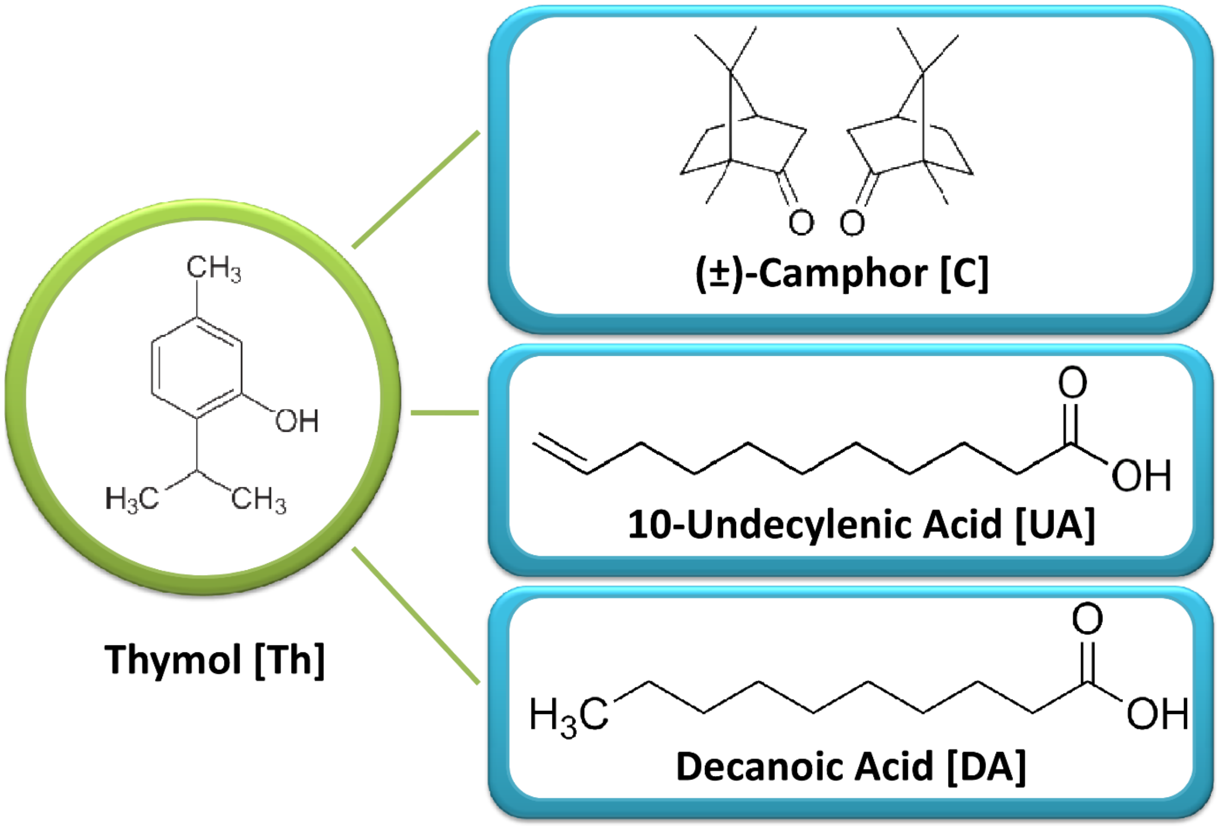

Figure 1 Chemical structures of compounds used to make deep eutectic solvents.

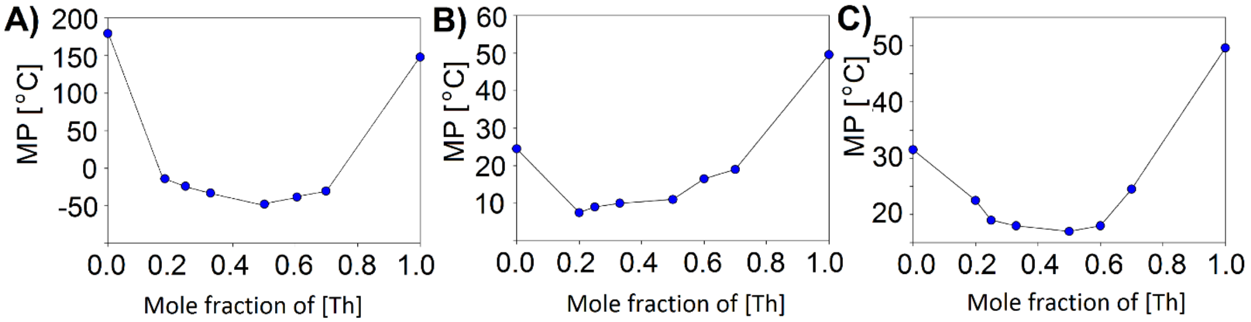

Figure 2 Solid-liquid phase diagrams of DESs: A) [Th][C], B) [Th][UA], C) [Th][DA].

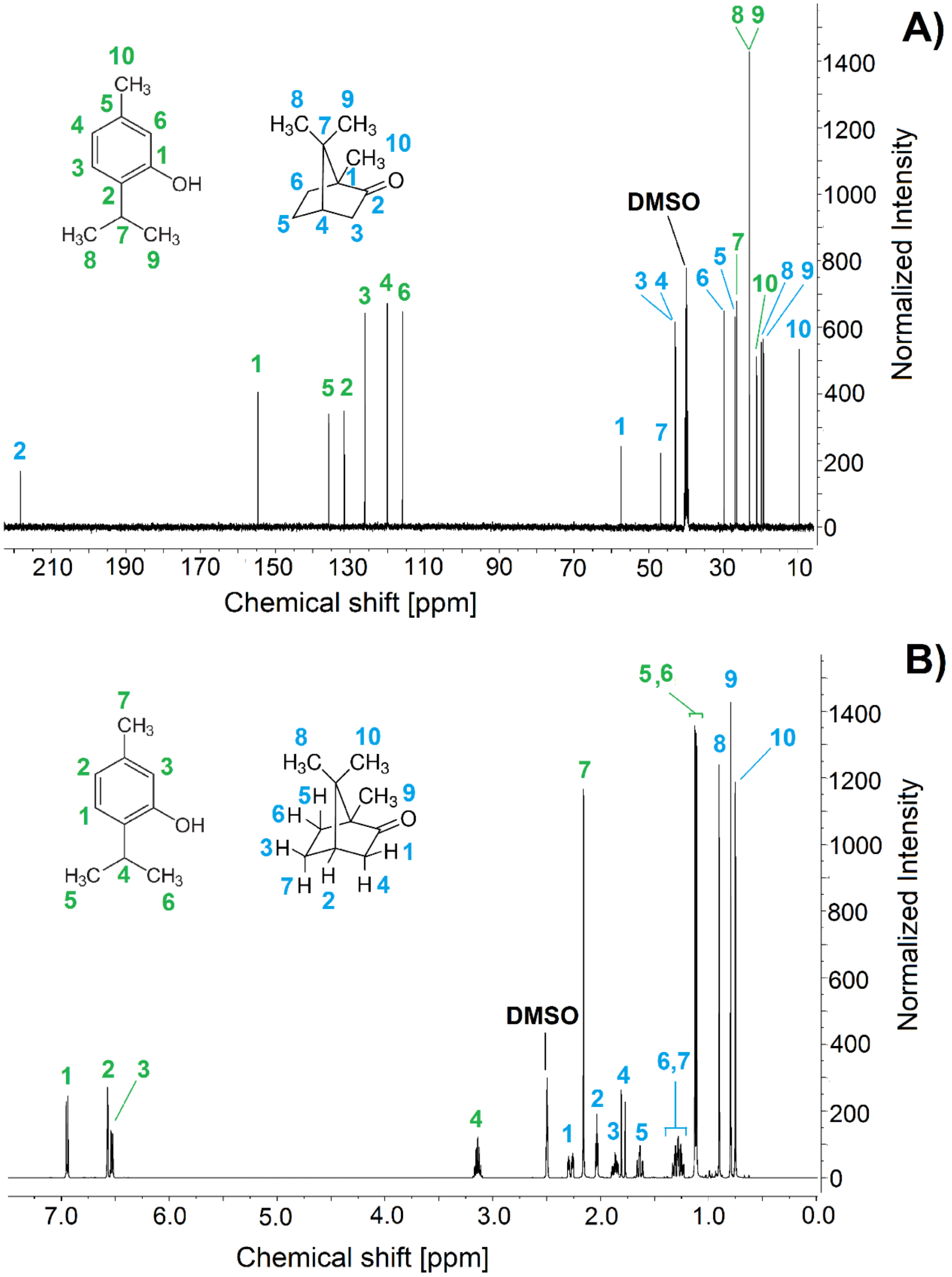

Figure 3 ^{13}C NMR spectrum (A) and ^{1}H NMR spectrum (B) of deep eutectic solvent [Th][C] 1:1 in DMSO.

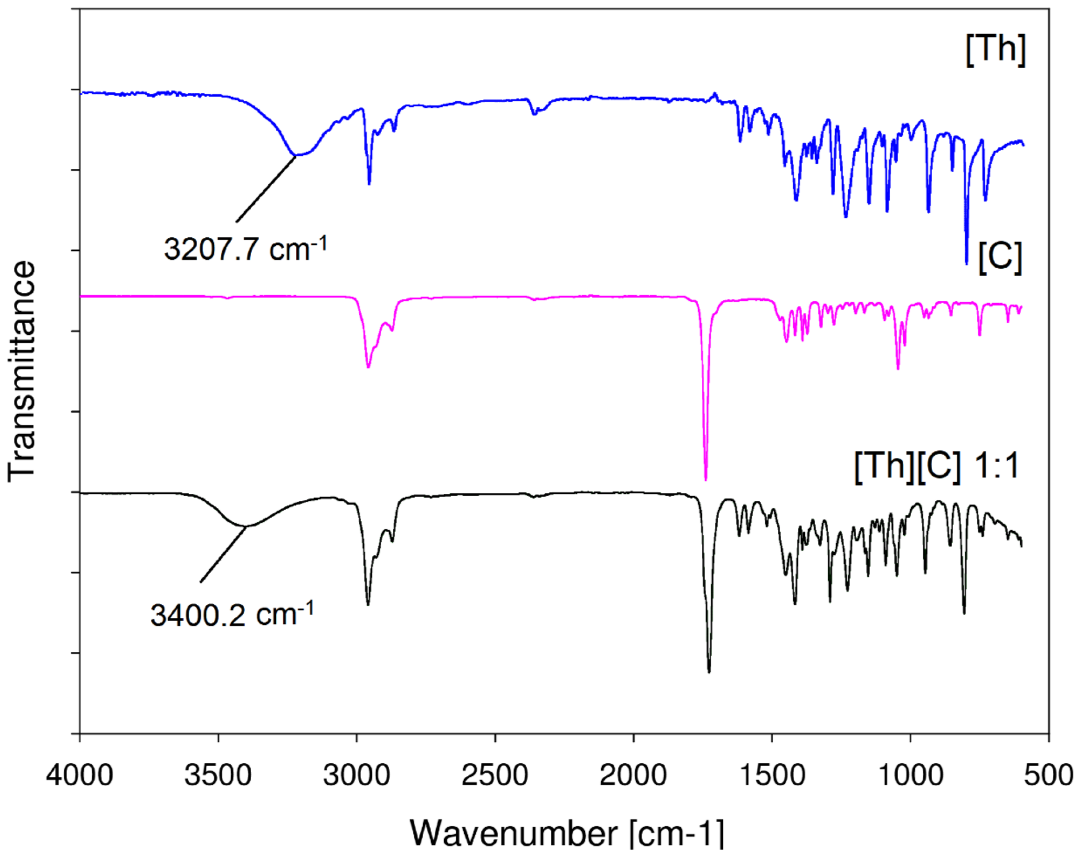

Figure 4 FT-IR spectrum of [Th][C] 1:1 and pure components.

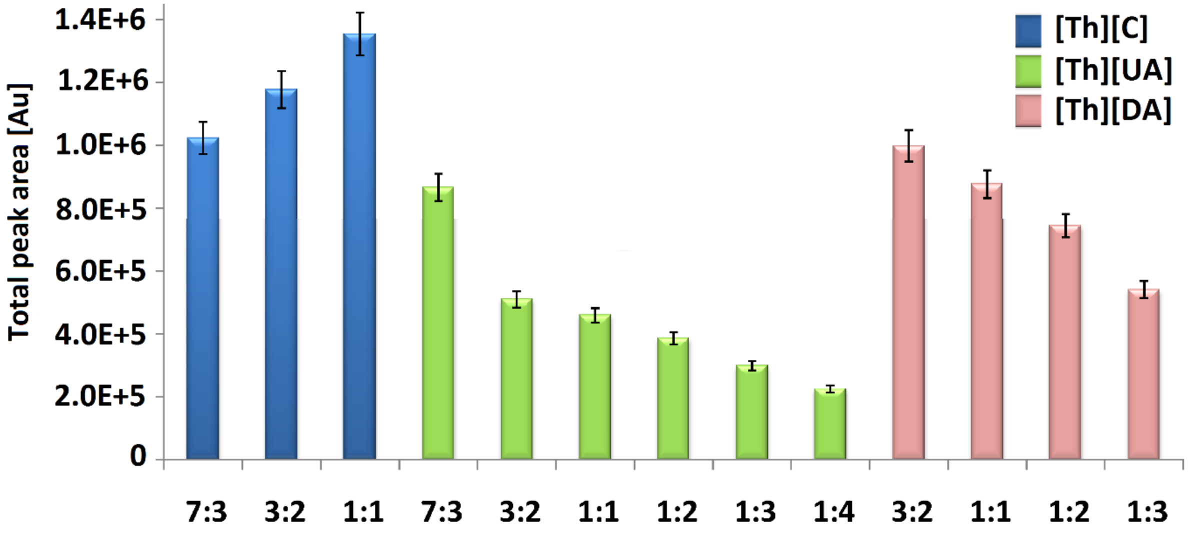

Figure 5 The effect of kind of DES on the PAH extraction yield.

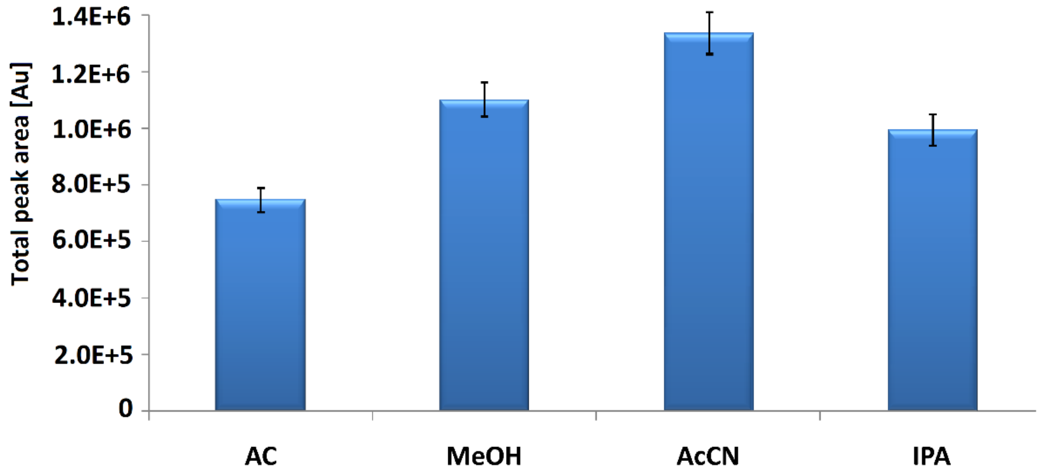

Figure 6 The effect of disperser solvent on the extraction yield.

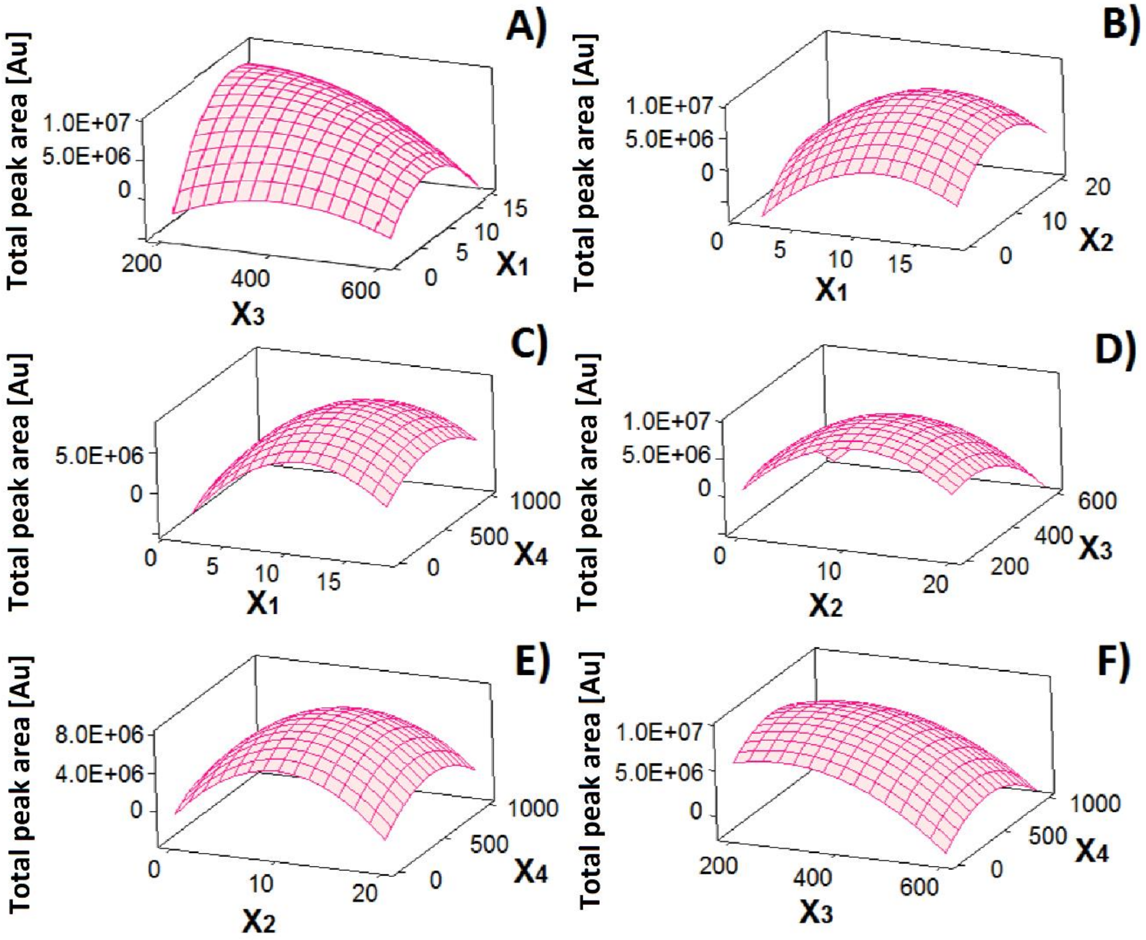

Figure 7 Response surface plots for PAH surface area dependence on: A) DES volumeand sonication time, B) sonication time and NaCl concentration, C) sonication time and AcCN volume, D) NaCl concentration and DES volume, E) NaCl concentration and AcCN volume, F) DES and AcCN volume.

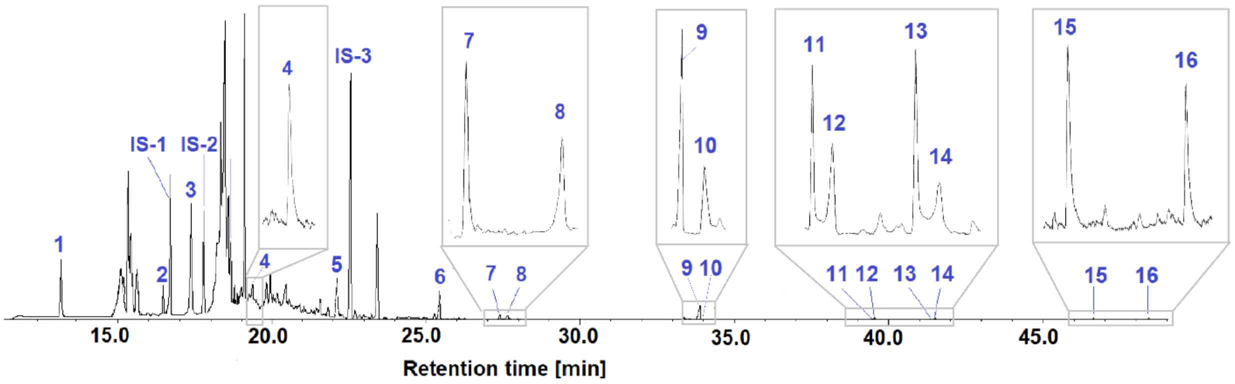

Figure 8 Chromatogram of real raw effluents. Identified compounds: 1) naphthalene, 2) biphenyl, 3) acenaphthylene, 4) fluorene, 5) anthracene, 6) 9-methyl anthracene, 7) fluoranthene, 8) pyrene, 9) benz[a]anthracene, 10) chrysene, 11) benzo[b]fluoranthene, 12) benzo[k]fluoranthene, 13) perylene, 14) benzo[a]pyrene, 15) indeno[1,2,3-cd]pyrene, 16) benzo[ghi]perylene, IS-1) 1-chloronaphthalene, IS-2) acenaphthene-d10, IS-3) anthracene-d10.