

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, \pm 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 $\mu\text{g/L}$ and 0.012 – 0.029 $\mu\text{g/L}$, 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 $\mu\text{g/L}$ 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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32 1. Introduction

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

46 Over the last few years, a number of classic extraction techniques have been replaced by so-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

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

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



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

77

78 **2. Experimental**

79 **2.1. Materials**

80 The following reagents were used in this study: thymol (purity $\geq 99.0\%$) and \pm camphor (purity \geq
81 95%) were purchased from Sigma-Aldrich (USA), decanoic acid (purity $\geq 98\%$) and 10-undecylenic acid
82 (purity $\geq 97\%$) were purchased from Merck (Germany). Solvents: methanol (MeOH), acetone (AC),
83 acetonitrile (AcCN), isopropanol (IPA) (purity $\geq 99.9\%$) were purchased from POCH (Poland). High purity
84 standards: naphthalene, biphenyl, acenaphthylene, acenaphthene, fluorene, fluoranthene,
85 phenanthrene, anthracene, 9-methyl anthracene, pyrene, benz[a]anthracene, chrysene, triphenylene,
86 benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene,
87 dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, benzo[b]chrysene and perylene (purity $\geq 98\%$) as well
88 as internal standards: 1-chloronaphthalene (purity $\geq 85\%$), acenaphthene-d10 (purity $\geq 99\%$) and
89 anthracene-d10 (purity $\geq 98\%$) were purchased from Sigma-Aldrich (USA).

90 **2.2. Real samples**

91 Effluents from the production of bitumen 20/30 (penetration grade 20-30 and softening point
92 55-63 °C, used as a paving grade bitumen suitable for road construction and repair) from vacuum
93 bottoms of crude oil Rebco:Kirkuk 65:35 m/m (mixture of Russian and Iraqi crude oils) were collected
94 behind a plate separator which separated condensed organic phase from aqueous phase. The aqueous
95 phase of raw effluents was investigated along with the effluents subjected to various chemical
96 treatments, including hydrodynamic cavitation (HC) aided by peroxone (mixture of O_3/H_2O_2) and HC
97 combined with sodium peroxydisulfate ($Na_2S_2O_8$). A detailed characteristic of the effluents was provided
98 in previous papers [[35]-[39]].

99 **2.3. Apparatus**

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



111 2.4. Procedures

112 2.4.1. Synthesis of DES

113 Deep eutectic solvents were synthesized by combining thymol with \pm camphor, 10-undecylenic
114 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
115 1:4. Next, the mixtures were stirred magnetically at 60 °C until homogeneous liquids were obtained. The
116 liquids were then left to cool spontaneously to room temperature.

117 2.4.2. Determination of physicochemical properties

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

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

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

129 2.4.3. Extraction procedure

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

137 2.4.4. Chromatographic analysis

138 Temperature program: 50 °C (2 min) – ramped at 10 °C/min to 190 °C (0 min) – ramped at 5
139 °C/min to 280 °C (5 min) – ramped at 5 °C/min to 310 °C (1 min); injection port temperature 330 °C;
140 injection volume 2 μL ; injection mode: splitless; detector temperature 310 °C; ion source temperature
141 (EI, 70 eV) 220 °C; GC/MS transfer line temperature 300 °C; the carrier gas was hydrogen (1 mL/min).

142 3. Results and discussion

143 3.1. Synthesis and physicochemical properties of DESs

144 If DESs are to be used as extraction solvents, they must be liquid at the extraction temperature.
145 The compounds used to prepare them (Figure 1) in their pure form are all solids at room temperature

146 with melting points for [Th], [C], [UA] and [DA] equal to 49.6, 179.8, 24.5 and 31.5 °C, respectively.
147 Among the investigated DESs, only some were liquids at ambient temperature, including [Th][C] at mole
148 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
149 [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
150 the investigated deep eutectic solvents are listed in Table 1. Solid-liquid phase diagrams of the DESs are
151 shown in Figure 2. The diagrams reveal a significant depression of DES melting points with respect to the
152 pure components. An especially large depression is observed for the DES formed from [Th] and [C] 1:1,
153 for which the MP is -44.0 °C.

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

158 In addition, in order to elucidate the interactions between the two components resulting in the
159 formation of DESs, FT-IR spectra were recorded. A comparison of FT-IR spectra of pure components and
160 the DESs formed is shown in Figure 4 and Figures S6-S7. The synthesis of deep eutectic solvents is
161 accomplished by the formation of hydrogen bonds between HBA and HBD. The location of the bonds
162 depends on the structure of the reactants. In ionic deep eutectic solvents, for example those formed by
163 the combination of choline chloride with phenols, hydrogen bonds are formed in the vicinity of -O-H
164 groups of phenols, which is indicated by a shift of the O-H band for a pure HBD towards lower
165 wavenumbers [[40]]. An inspection of FT-IR spectra of all the investigated deep eutectic solvents also
166 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
167 [Th][C], [Th][UA], and [Th][DA], respectively. In addition, the spectra of DESs formed from thymol and
168 carboxylic acids reveal a characteristic shift of the bands corresponding to stretching vibrations of the
169 carbonyl group towards higher wavenumbers: from 1699.1 cm⁻¹ to 1722.6 cm⁻¹ and from 1698.2 cm⁻¹ to
170 1725.5 cm⁻¹ for [Th][UA] and [Th][DA], respectively, which indicates the formation of new hydrogen
171 bonds in the vicinity of the -COOH group. A similar behavior was also observed in DESs composed of DL-
172 menthol and carboxylic acids [[41]].

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

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

182 3.2. Optimization of extraction conditions

183 Optimization of extraction conditions using deep eutectic solvents as the extractants was carried
184 out for several PAHs, including fluorene, phenanthrene, pyrene and benzo[a]pyrene which were selected
185 as representative PAHs. Most of the published research indicate very similar impact of optimized



186 parameters on the extraction efficiency of all PAHs [[11]-[12],[14]-[15]]. The extraction efficiency was
187 estimated from total peak areas of the selected analytes.

188 **3.2.1. Kind of DES**

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

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

202 **3.2.2. Kind of disperser solvent**

203 Several disperser solvents were tested in the investigations: AC, MeOH, AcCN and IPA. The
204 following conditions were used: 300 μL [Th][C] 1:1, 700 μL of disperser solvents, pH 7, 0% w/v NaCl, and
205 15-min sonication. The highest extraction yield of PAHs was obtained with AcCN. The kind of disperser
206 solvent has only a slight effect of extraction yield which is in agreement with a previous work [[7],[42]-
207 [45]]. The effect of disperser solvent on the extraction yield is depicted in Figure 6.

208 **3.2.3. Central Composite Design**

209 The central composite design (CCD), also called the Box-Wilson design [[46]], was selected for
210 the optimization of major parameters potentially affecting the extraction yield. CCD is a five-level
211 factorial design consisting of edge points (+1 and -1) corresponding to the upper and lower limits of the
212 investigated factor, the star points ($-\alpha$ and $+\alpha$) and the center point. The following extraction parameters
213 were selected: sonication time (2-18 min), salting out effect (0-20% NaCl), DES ([Th][C] 1:1) volume (200-
214 600 μL) and disperser solvent (AcCN) volume (0-1000 μL). The exact values for the five levels
215 are compiled in Table 2 while the CCD matrix and the results of PAH extraction are listed in Table S1.

216 The results were analyzed by ANOVA and regression in order to create an appropriate model and
217 select statistically important parameters (Table 3). In ANOVA, the values of statistical parameters F and p
218 were used as criteria at a 95% confidence level. All the parameters for which the p-value was less than
219 0.05 were considered as statistically significant and having a substantial effect on the model. The
220 developed model was found to be statistically significant due to the p-value < 0.0001 and F-value equal
221 to 50.35 (Table 3). The values of lack of fit (p-values greater than 0.06) were considered insignificant. The
222 response equation (1) obtained for the experimental results is shown below:

$$\begin{aligned}
 223 \quad Y = & -28117320 + 2958279 X_1 + 1334994 X_2 + 71672.8 X_3 + 7243.8 X_4 - 93581.4 X_1^2 - 58484.5 X_2^2 - 81.8 X_3^2 \\
 224 \quad & - 12.4 X_4^2 - 2063.9 X_1 X_3 \qquad \qquad \qquad (1)
 \end{aligned}$$

225 where Y is the total peak area of the peaks of selected PAHs. The equation explains the effect of the
 226 investigated parameters on the PAH extraction yield. The developed model has a large coefficient of
 227 determination ($R^2 = 97.92$) along with the values of predicted- R^2 and adjusted- R^2 , equal to 89.07 and
 228 95.97%, respectively, which demonstrates a good fit of the model to the experimental values and the
 229 possibility of prediction of responses for new data.

231 3.2.4. Interaction effect

232
 233 In order to facilitate selection of the optimum values of volumes of DES and AcCN, NaCl
 234 concentration and sonication time, the results were plotted as response surfaces (Figure 7). The results
 235 demonstrated that the most pronounced effect on the extraction yield have DES volume and sonication
 236 time (p -value < 0.002). Figure 7 (A,D,F) reveals that an increase in DES volume from 200 μ L to 600 μ L is
 237 accompanied by a significant decrease in the extraction yield. An increase in the extractant volume
 238 results in an increase in volume of the extract, thus lowering the concentration of analytes and their
 239 peak areas (for the same injection volume). Extending sonication time from 2 to 14 min improved the
 240 extraction yield while further extension of time deteriorated the extraction yield (Figure 7 A-C).
 241 Extending the time of sonication is particularly advantageous when using small amounts of extraction
 242 solvent (Figure 7A). Ultrasounds facilitate dispersion of the extractant thus improving the extraction
 243 yield; however, extensively long sonication results in degradation of PAHs in aqueous matrices [[47]].
 244 This is due to acoustic cavitation taking place in aqueous media which can result in degradation of a
 245 variety of organic compounds [[48]-[49]].

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

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

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

269 **3.3. Validation of procedure**

270 Quantitative analysis was based on the internal standard method using three internal standards:
271 1-chloronaphthalene, acenaphthene-d10 and anthracene-d10. Nine-point calibration curves were
272 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
273 chromatogram of a mixture of standards at a concentration of 1 µg/mL. Two characteristic mass-to-
274 charge ratio (m/z) values were determined for each analyte, *i.e.*, target ion, which was used to determine
275 peak areas needed for calibration curves and the determination of concentrations of PAHs in real
276 samples, and the qualifier ion used for confirmation of identification of PAHs present in samples. A
277 compilation of retention times and characteristic ions is shown in Table S2.

278 The LOD and LOQ values for the majority of PAH analytes ranged from 0.0039 to 0.0098 µg/L and
279 0.012 to 0.029 µg/L, respectively. Higher LOD and LOQ values were obtained for 9-methyl anthracene
280 (0.037 and 0.11 µg/L) and for benzo[ghi]perylene (0.021 and 0.063 µg/L). Additionally, a wide linear range
281 and good coefficients of determination (R^2) ranging from 0.9989 to 0.9999 were obtained for all of the
282 analytes. Detailed results are presented in Table 4.

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

293 A comparison of the developed procedure with other procedures for the determination of PAHs
294 revealed that the proposed procedure has similar LOD and analyte recovery compared to the existing
295 procedures while offering a significant improvement in precision. In comparison with solid phase
296 extraction (*i.e.*, SPME, SPE) as well as solvent microextraction procedures, *i.e.*, solvent bar
297 microextraction (SBME) or SDME, the developed procedure has a shorter sample preparation time
298 [[12],[14]-[16]]. Furthermore, an advantage of the proposed procedure is the use of a nontoxic
299 extracting solvent, offering an alternative to the most popular procedures using chlorinated solvent to
300 extract PAHs from aqueous samples [[7],[13]-[14]]. A comparison of the new procedure with the one
301 based on Emulsification Liquid-Liquid Microextraction using a Deep Eutectic Solvent (ELLME-DES)
302 revealed that the former is characterized by lower LOD values [[53]].



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

304 **3.4. Analysis of real samples**

305 The developed procedure was used for the analysis of samples of effluents from the production
306 of bitumens, both raw and subjected to treatment by hydrodynamic cavitation (HC) aided by peroxone
307 or Na₂S₂O₈. The analytes were identified based on the ratio of intensities of characteristic ions listed in
308 Table S2, taking the confidence interval of ± 15 %, and also based on the values of retention times ± 0.1
309 min.

310 In samples of raw effluent, 16 PAHs analytes were identified at concentrations ranging from 0.12
311 to 46.2 µg/L, the most abundant being naphthalene, biphenyl, acenaphthylene, anthracene,
312 benz[a]anthracene and 9-methyl anthracene (Figure 8). In addition, another derivative of 9-methyl
313 anthracene with a substituent in a different position in the ring was tentatively identified in raw effluent
314 as indicated by a peak with a retention time of 25.46 min, which has the same characteristic ions with
315 similar intensities. The results obtained for samples of treated effluent reveal a much better
316 effectiveness of degradation of the method HC/peroxone, which resulted in a complete or almost
317 complete degradation of the majority of the analytes. In contrast, the effluent treated by the HC/Na₂S₂O₈
318 process, PAHs were oxidized to a much lesser extent and the effectiveness of treatment did not exceed
319 29.85%. Nevertheless, in both cases the effectiveness of degradation of PAHs was higher for the analytes
320 having a lower boiling point.

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

323 **4. Summary**

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

335 The procedure was used in the investigations of effectiveness of treatment of effluents from the
336 production of bitumens, which have a matrix rich in various types of organics. The presence of a number
337 of PAHs at concentrations ranging from 0.12 to 46.2 µg/L in the effluent indicates the need for effective
338 treatment methods [[54]-[55]]. A comparison of two treatment methods based on hydrodynamic
339 cavitation aided by different oxidants, including peroxone and Na₂S₂O₈ reveal a much better degradation
340 yield of the method based on the O₃/H₂O₂ mixture.

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480 **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

481 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 –
 482 mole fraction of the second component of DES, M_2 – molar mass of the second component [g/mol], MP – melting point [°C].
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488 **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 [μ L]	200	300	400	500	600
(X ₄) Volume of AcCN [μ L]	0	250	500	750	1000

489 ¹⁾ k – number of variables = 3
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498 **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

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515 **Table 4** Analytical characteristics of the developed procedure.

Compound	Calibration curve	R ²	LOD [$\mu\text{g/L}$] ¹⁾	LOQ [$\mu\text{g/L}$] ²⁾	Linear range [$\mu\text{g/L}$] 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: $LOD = 3 \cdot S/N$ (S – signal, N – noise),

²⁾ The limit of quantitation (LOQ) was calculated from: $LOQ = 3 \cdot LOD$,

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 [$\mu\text{g/L}$], x_{ist} – internal standard concentration [$\mu\text{g/L}$] [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.

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550 **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	-

- 551 ¹⁾ Recovery (R) determined after addition of analytes to deionized water. (Recovery (R) was calculated from: $R[\%] = \frac{C_{\text{quant}} - C_0}{C_{\text{expect}}} \cdot 100\%$,
552 where: C_{quant} – found analyte concentration in spiked sample [µg/L], C_{expect} – analyte concentration added as spike [µg/L], C_0 – found
553 analyte concentration in non-spiked samples [µg/L]).
554 ²⁾ Recovery (R) determined after addition of analytes to real effluent.
555 ³⁾ Intraday RSD determined after addition of analytes to deionized water and 4 analyses on the same day.
556 ⁴⁾ Interday RSD determined after addition of analytes to deionized water and 6 analyses on 3 consecutive days.

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559 **Table 6** Comparison of the developed procedure with other procedures for the determination of PAHs found in
560 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

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



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

Compound	Concentration ± SD [µg/L]					
	HC/peroxone			HC/Na ₂ S ₂ O 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

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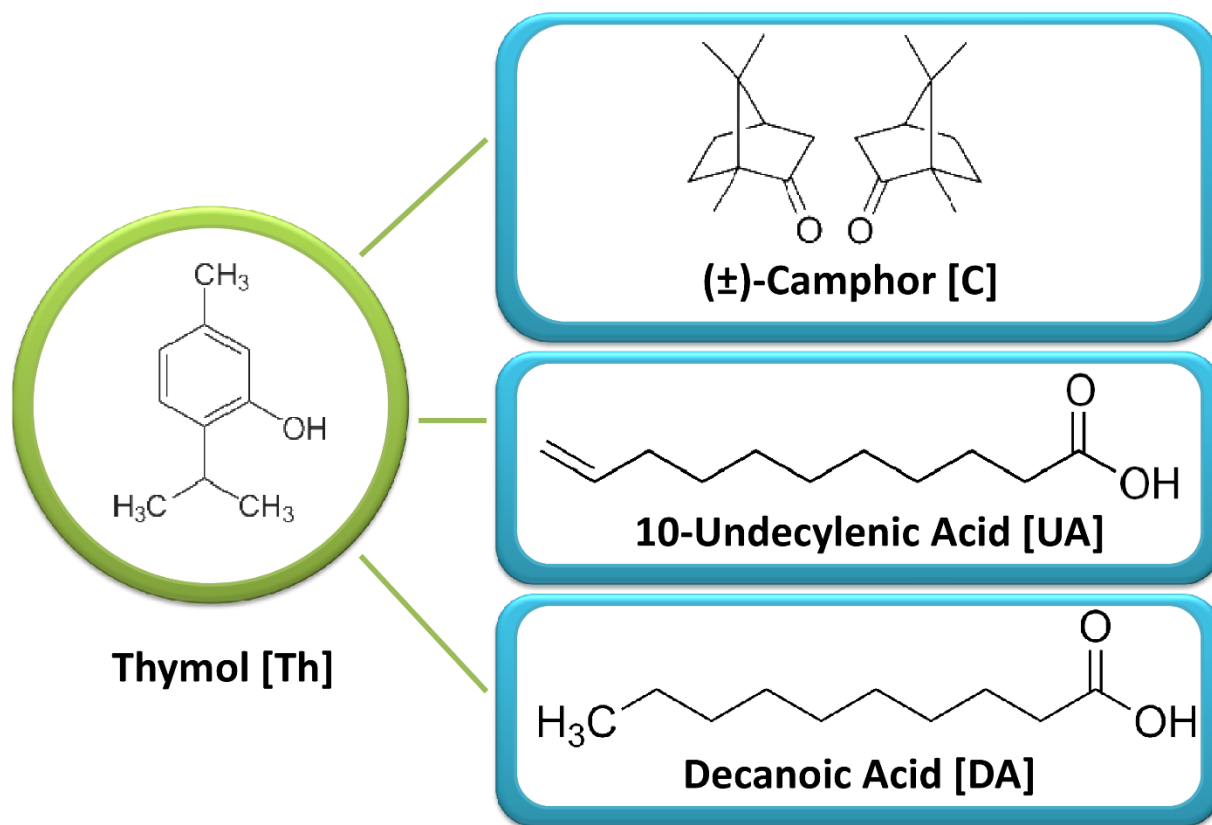
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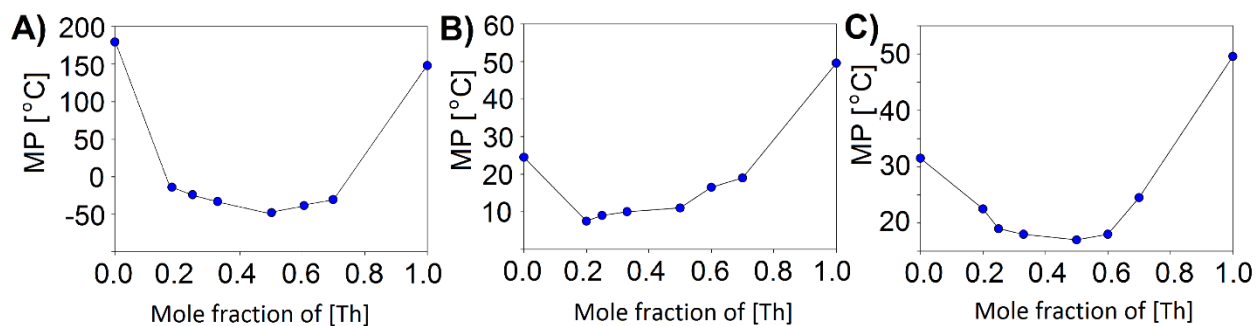




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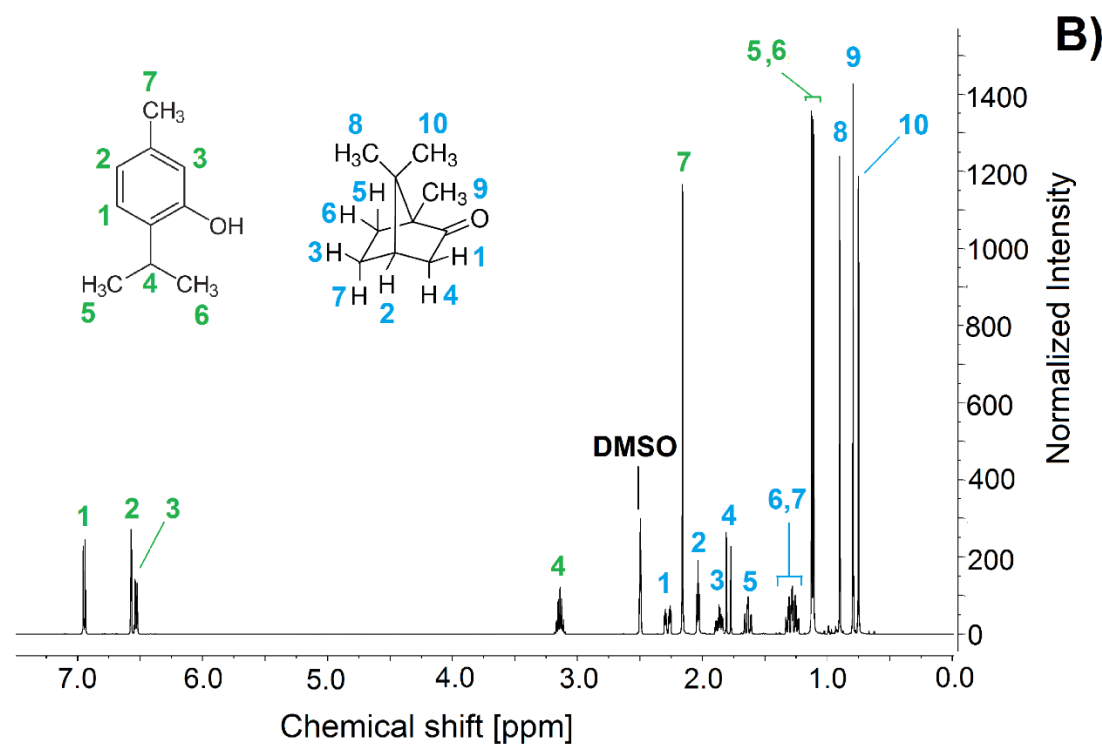
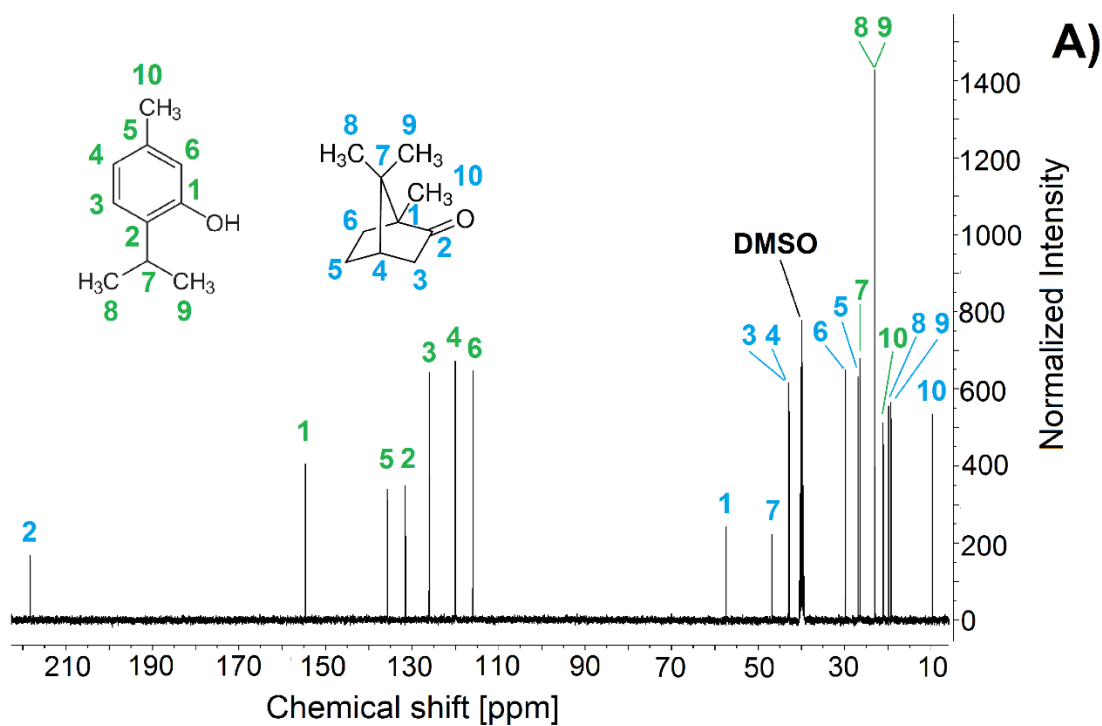
581 **Figure 1** Chemical structures of compounds used to make deep eutectic solvents.

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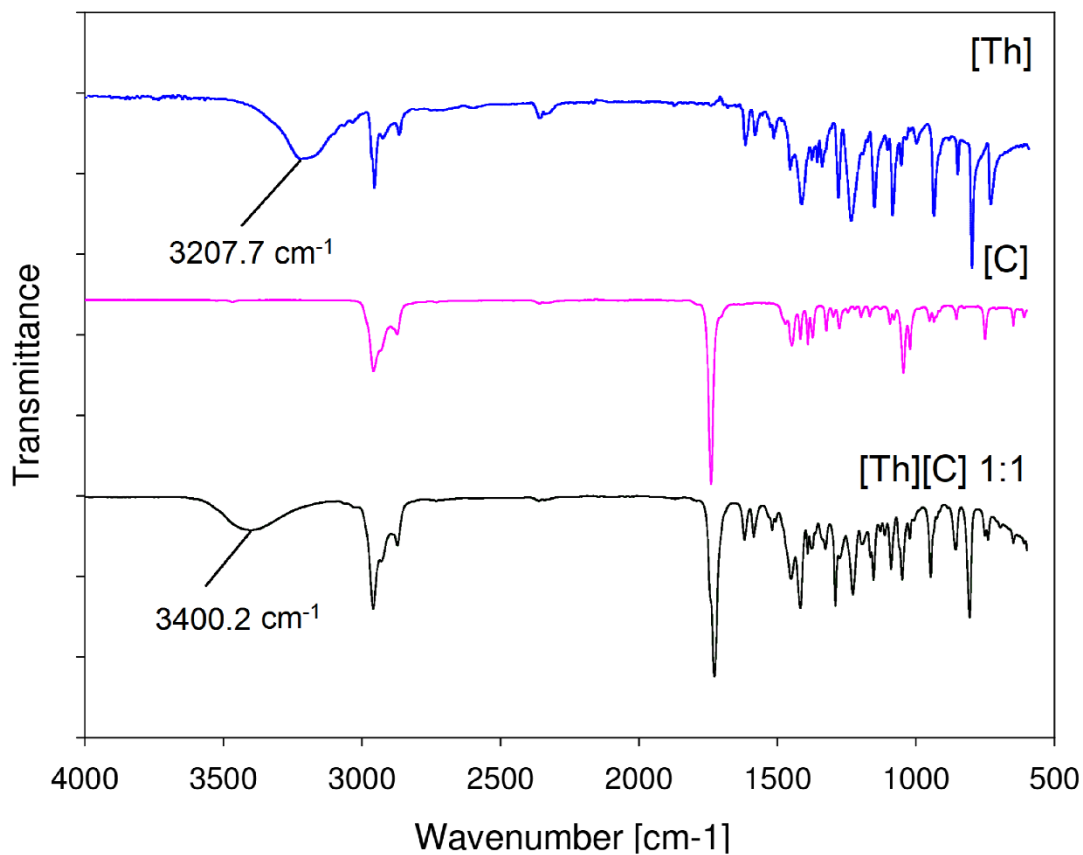


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584 **Figure 2** Solid-liquid phase diagrams of DESs: A) [Th][C], B) [Th][UA], C) [Th][DA].

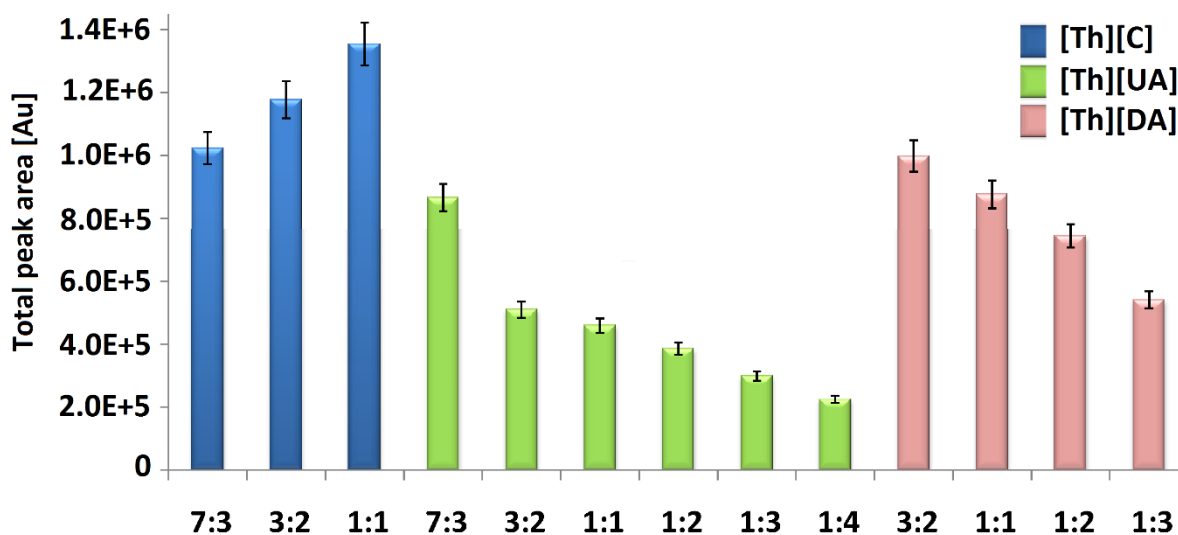


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 586 **Figure 3** ^{13}C NMR spectrum (A) and ^1H NMR spectrum (B) of deep eutectic solvent [Th][C] 1:1 in DMSO.



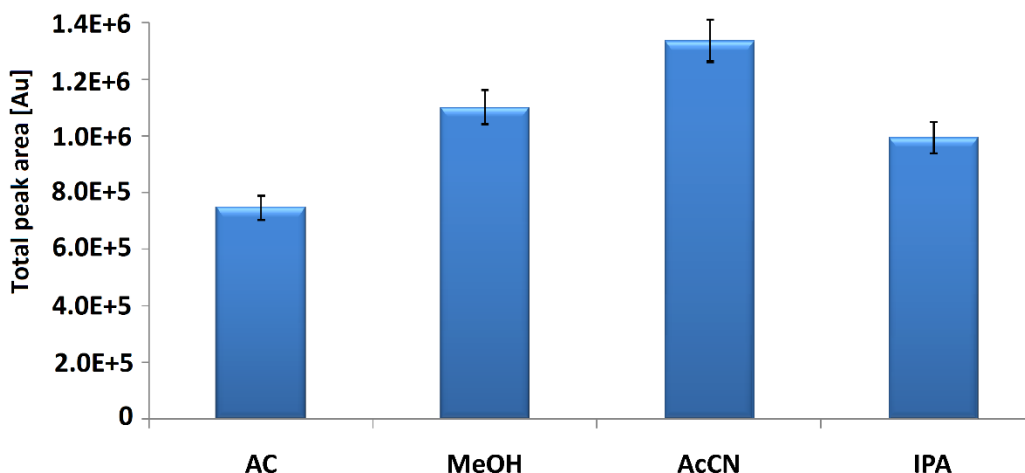
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588 **Figure 4** FT-IR spectrum of [Th][C] 1:1 and pure components.



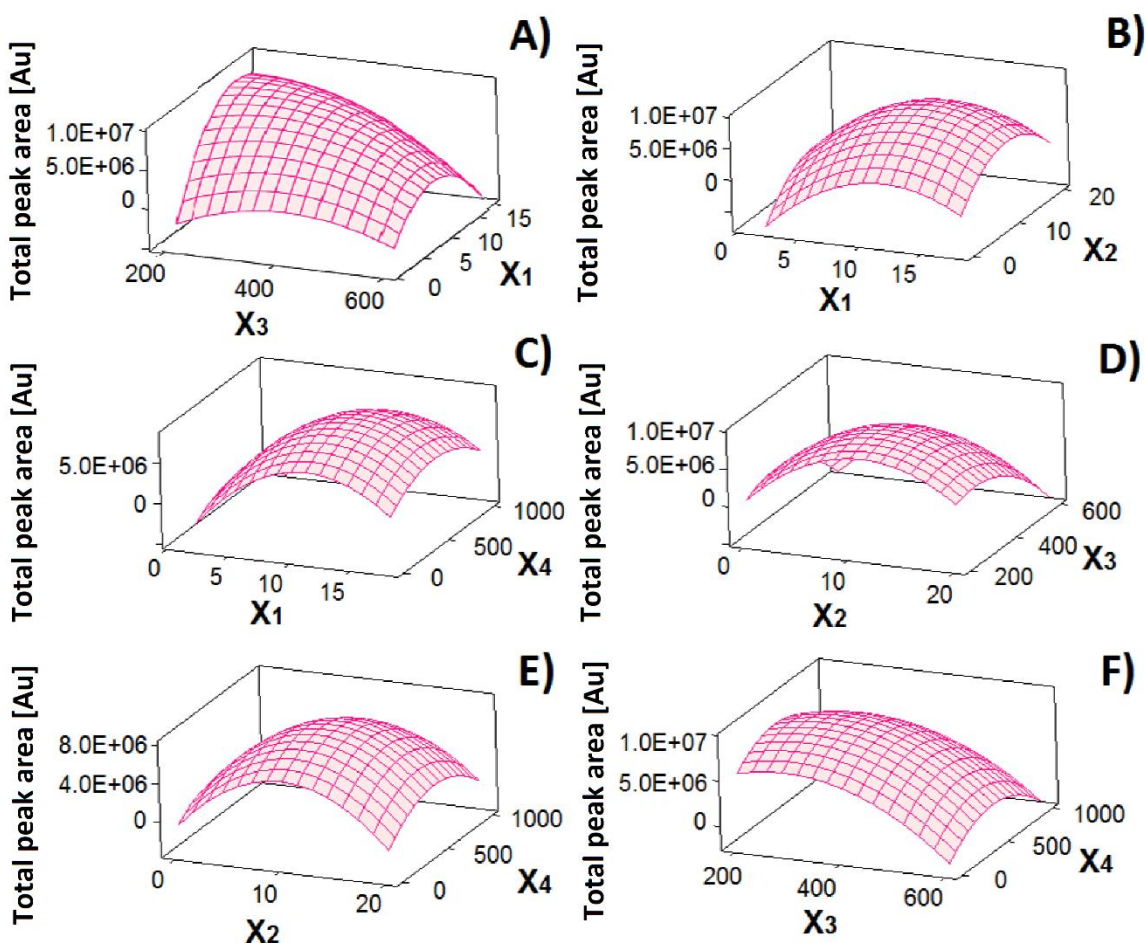
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590 **Figure 5** The effect of kind of DES on the PAH extraction yield.



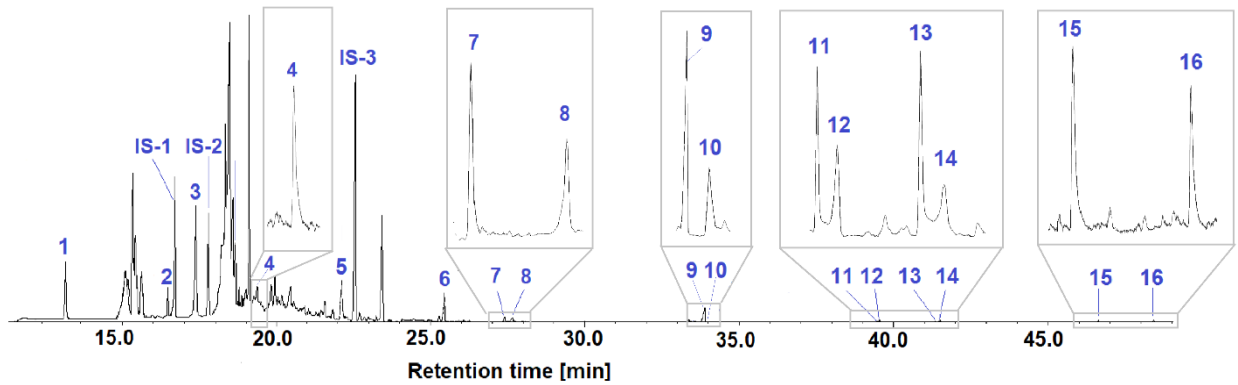
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592 **Figure 6** The effect of disperser solvent on the extraction yield.



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



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