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Optimization of deep eutectic solvent based liquid phase microextraction of PAHs in environmental samples using response surface methodology

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Abstract: The presence of polycyclic aromatic hydrocarbons (PAHs) in wastewater poses significant health risks. To address this, a novel deep eutectic solvent-based ferrofluid (DES-ferrofluid) was developed for liquid phase microextraction with back extraction (LPME-BE) to detect PAHs. The DES-ferrofluid was characterised for its physicochemical properties and morphology using FTIR, VSM and SEM-EDX. Key parameters in the LPME-BE process were optimised using response surface methodology (RSM) based on a Box–Behnken design (BBD). Optimal conditions included 15 mg of tetraethyl orthosilicate-coated magnetic nanoparticles (MNPTEOS), 25 μL of DES 1 (caprylic acid and lauric acid), 800 mg NaCl and a 10 min extraction time. Analysis of variance (ANOVA) confirmed strong alignment between experimental data and the model, with an R^2 of 0.8799 and adj. R^2 of 0.7395. The method achieved limits of detection (*LOD*s) of 0.4–1.7 ng mL^{-1} and limits of quantification (*LOQ*s) of 1.33–5.67 ng mL^{-1}. Recoveries for spiked samples ranged from 75.78 to 118.65 %, with *RSD* < 15 %. This DES-ferrofluid LPME-BE method with BBD optimization shows promise as an effective alternative for PAH detection in wastewater samples.

Keywords: ferrofluid; fatty acid-based deep eutectic solvent; green microextraction; Box–Behnken design.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are among the most common organic pollutants disposed of into the environment, resulting in serious environmental concerns. PAHs are chemical compounds with two or more fused benzene rings insoluble in water. PAHs with fewer than four rings are considered light

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PAHs, while PAHs with more than four rings are considered heavy PAHs. Heavy PAHs are more stable and dangerous than light PAHs.[1] PAHs result from thermal deterioration, which occurs due to the incomplete combustion of organic materials and the geochemical creation of fossil fuels. Power plants, domestic heating, waste incineration, industrial activities and, most importantly, automotive exhaust emissions are the principal sources of anthropogenic pollution.

Given by the occurrence and adverse side effects of PAHs, the development of effective analytical methods for identifying PAHs in samples is highly desired. PAHs analysis is typically conducted in various samples using chromatographic techniques such as high-performance liquid chromatography[2,3] and gas chromategraphy.[4,5] Despite their high sensitivity, the presence of PAHs at low concentrations in samples and complex matrices has hindered their direct application. A sensitive and selective sample pretreatment technique is required to clean up and pre-concentrate the analytes before chromatographic analysis.[1,6]

Liquid-phase microextraction (LPME), a novel method for complex matrices pretreatment, can be considered an environmentally friendly, simple, easy-to-operate and highly sensitive method for preconcentration. A miniaturised preparation method allows trace determination of target compounds in complex matrices.[7] Recently, green solvents, namely ionic liquids and deep eutectic solvents, have been significantly adopted in LPME as a substitute for hazardous solvents. The utilisation of deep eutectic solvents (DES) has garnered heightened interest in LPME.[8] DES is a combination of two compounds: a hydrogen-bonding donor and a hydrogen-bonding acceptor, which form a eutectic mixture. Deep eutectic solvents (DES) exhibit characteristics similar to ionic liquids (IL), such as high thermal stability, viscosity and high tunability, rendering them a good substitute for hazardous solvents.[9,10] Owing to their distinctive physicochemical characteristics, DES are advantageous in diverse extraction processes, such as serving as coating agents or carrier liquids in producing ferrofluids.[4,11,12]

Ferrofluid liquid-phase microextraction (LPME) is a novel and environmentally friendly method that utilizes magnetic nanoparticles (MNPs) dispersed in a carrier fluid. This technique does not require centrifugation or advanced equipment, allowing for rapid phase separation with the aid of an external magnetic field. Surface coating techniques that use surfactants or ionic charges to preserve colloidal stability are employed to stop MNPs sediment and clumping together due to their high reactivity. Because of their low toxicity, affordability, environmental sustainability and capacity to modify the physicochemical characteristics of ferrofluids for improved extraction performance, deep eutectic solvents (DESs) have become the preferred choice for the carrier fluid.

Numerous experiments have shown that DES-based ferrofluids can extract analytes from complicated matrices. Dil *et al.* developed a hydrophobic octanoic acid–DL–menthol DES ferrofluid to determine the amount of doxycycline in urine,

blood plasma and milk.[13] This method achieved excellent extraction efficiency using 150 μL of ferrofluid and 7 min of vortex. Zarei *et al.* created a magnetic clay nanoparticle coated with a menthol–decanoic acid DES to remove explosives from water, producing recovery rates ranging from 88 to 104 %.[14] Similarly, Jouyban *et al.* extracted 16 PAHs from urine and saliva using a toner-based ferrofluid modified with choline chloride–stearic acid DES, with recoveries ranging from 61 to 84 %.[4] In a different application, PAHs were extracted from grilled meat using a ternary DES (phosphocholine:menthol:decanoic acid) ferrofluid, which produced excellent recovery and selectivity.[12] Mohebbi *et al.* extracted pesticides such as diazinon, deltamethrin and fenpropathrin using a choline chloride–stearic acid ferrofluid, achieving recovery rates of 82–94 % and enrichment factors of 1643––1884.[15] To extract PAHs from coffee, Fan *et al.* created a ternary DES (menthol––borneol–camphor) ferrofluid, which achieved recovery rates of 91–121 % and detection limits of 0.31–5.9 ng L^{-1}.[16]

Tamoxifen and its active metabolites were extracted from plasma more recently by Abarbakouh *et al.* using a ferrofluid dispersive solid-phase microextraction in conjunction with LC–UV.[17] They achieved low detection limits (0.2–200 μg/L), precision (intra-day ≤ 7.5 %; inter-day ≤ 11.2 %), and accuracy (94–105 %). For the quantification of remdesivir in human plasma, Morovati *et al.* used a DES––IL ferrofluid microextraction approach, which produced a linear range of 0.5–500 μg L^{-1}, low detection limits (0.2 μg L^{-1}), and good precision (intra-day ≈ 9 %; inter-day ≈ 17 %) and accuracy (90–107 %).[18] Due to their avoidance of centrifugation and external dispersive solvents, these techniques align with the principles of green analytical chemistry. Therefore, they are ideal for regular pharmaceutical tests, therapeutic monitoring, and clinical pharmacokinetic research.

Despite significant advancements, little is known about the long-term stability, reusability and environmental impact of ferrofluids based on deep eutectic solvents (DES). There are also few studies investigating their compatibility with automated platforms, potential toxicity profiles, and comparative performance across different analyte classes and matrices. Further research is needed to fully demonstrate the scope, reliability and sustainability of ferrofluid-based microextraction procedures.

Several important parameters, such as the type of solvents, pH and solvent volume, must be optimised to establish optimal extraction procedure conditions. Previous research has presented the “trial and error” strategy, in which one parameter is changed at a time while other parameters must remain constant to achieve effective analyte separation.[19,20] Due to the enormous number of experimental steps and organic solvent consumption, testing the parameters at such levels is time-consuming and cost-ineffective, resulting in unsatisfactory results. Conversely, this approach does not demonstrate the interactive response at the extreme values of independent parameters.[20]

Hence, response surface methodology (RSM) is critical in determining the ideal combination of parameters that produces the best chromatographic responses, such as sensitivity, recovery, and peak area. RSM is a statistical technique that is a significant tool for modelling and analysing the impacts of various process parameters. The underlying principle is to get the best conditions by doing the fewest trials possible and estimating interactions between the independent variables. Several previous studies have combined sample pretreatment methods with statistical analysis tools to optimise their experimental process by reducing the number of experiments while improving the accuracy of the results. The use of RSM to optimise experimental conditions has been widely used in various extraction techniques, including PAHs determination[21,22] and organo-phosphate pesticide determination.[23–25] Response surface methodology (RSM), which includes central composite design (CCD), Box–Behnken design (BBD) and Doehlert design, is used to optimise the most important independent parameter and determine the optimum amount of each factor.[26] BBD is primarily a factorial design used in RSM to examine the quadratic response surface and construct second-order polynomial models. BBD is a spherical, three-level fractional factorial design comprised of a central point and the middle points of the circle's circumscribed edges of the sphere.[26,27] BBD needs fewer experimental runs than other factorial designs, making it more practical and cost-effective.[26,28]

Further research is imperative to understand the effect of independent variables on the LPME-BE technique utilising DES-based ferrofluids with Box–Behnken design. Therefore, this work aims to investigate RSM experimental design approaches for optimising independent parameters and validate the method for quantifying PAHs determination using the DES-based ferrofluids LPME method. In addition, DES-based ferrofluid LPME with back extraction was employed to analyse extracted PAHs in various environmental samples.

EXPERIMENTAL

Chemicals and reagents

All fatty acids (caprylic acid (≥ 99.0 %), capric acid (≥98.0 %), pelargonic acid (≥97.0 %) and lauric acid (≥ 98.0 %) were purchased from Sigma–Aldrich. Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and ammonium iron (II) sulphate hexahydrate ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$) were obtained from QRec (Auckland, New Zealand). Tetraethyl orthosilicate (≥ 98.0 %) was purchased from Merck. HPLC-grade acetonitrile and methanol were purchased from QRec (Auckland, New Zealand). Analytical grade solvents such as ammonia solution (28 %), hexane, dichloromethane, ethanol and isopropyl alcohol were also supplied by QRec (Auckland, New Zealand). Ultrapure water was prepared by the Millipore water purification system (Molsheim, France).

Polycyclic aromatic hydrocarbons (PAHs) analytical standards (naphthalene, (≥ 99.7 %), biphenyl, (≥ 98.0 %), acenaphthylene (≥ 99.0 %), anthracene (≥ 98.0 %) and pyrene (≥ 98.0 %) were provided by Sigma–Aldrich. The individual stock solution for each PAH (1000 μg mL^{-1}) in acetonitrile and stored in dark at 4 °C. The working standard solution containing the PAH

mixtures of a concentration of 10 μg mL^{-1} was prepared by appropriate dilution of the stock solution with acetonitrile.

Synthesis of magnetic nanoparticles (MNP)

The magnetic nanoparticles (MNP) was prepared using previous methods.[29] About 4.8 g of $(NH_4)_2{\cdot}Fe(SO_4)_2{\cdot}6H_2O$ and 9.6 g of $FeCl_3{\cdot}6H_2O$ were mixed with 120 mL of deionised water. The mixture was stirred in a pre-heated solution (60 °C) for 5 min in an N_2 atmosphere. The ammonia solution was then added dropwise and stirred for 1 h. The solid products were recovered using an external magnet and rinsed with excess deionised water until the pH reached neutral and dried in an oven for 24 h.

The magnetic nanoparticles with tetraethyl orthosilicate (MNPTEOS) was produced by sonicating the synthesised MNP in a 200 mL mixture of ethanol and deionised water (1:1 volume ratio) for 20 min.[12] Ammonia (15 mL) and tetraethyl orthosilicate (TEOS, 6.3 mL) were gradually added into the mixture. The mixture was then agitated for 8 hours in a pre-heated solution (50 °C) under an N_2 atmosphere. The solid products were gathered using an external magnet after cooling at room temperature, washed thrice with ethanol and deionised water (30:70 volume ratio) and dried in the oven for 24 h.

Synthesis of deep eutectic solvent (DES)

The selected fatty acid-based deep eutectic solvent (DES) synthesis was carried out using a previously published method.[30]

Preparation of DES-based ferrofluids

About 15 mg of MNPTEOS was mixed into 25 μL of DES 1 and sonicated for 15 min to produce stable DES-based ferrofluid (MNPDES). The MNPDES was rinsed with deionised water to eliminate unreacted compound.[12]

Characterisation

The vibrational frequencies of each functional group in all MNP, MNPTEOS and MNPDES were analysed using Fourier transform-infrared spectroscopy (FTIR). The study was conducted with a Perkin Elmer 2000 instrument (Waltham, USA). The FTIR spectrum has been acquired in the range of 400–4000 cm^{-1} to analyse and determine the unique chemical properties within the sample. The magnetic properties of the synthesised MNP, MNPTEOS and MNPDES were analysed using a Lake Shore 4700 series VSM (Westerville, OH, USA). The magnetic field was applied within the range of –8000 to 8000 G. The Quanta™ 650 FEG SEM (Hillsboro, OR, USA) was used to analyse the surface morphology and elemental dispersion of the MNP, MNPTEOS and MNPDES. EDX analysis offers elemental analysis to characterise the components present in the sample. The EDX spectra reveal the elemental composition and distribution found in MNP, MNPTEOS and MNPDES. The analysis was conducted using an operating voltage of 10.00–15.00 kV, a magnification of 100,000×, and a working distance (*WD*) of 9.7––9.8 mm.

Liquid phase microextraction with back extraction (LPME-BE) procedure

Initially, 25 μL of MNPDES was added into a 20 mL sample bottle followed by 5 μg mL^{-1} of PAHs mixtures and 800 mg of NaCl, and vortexed for 10 min using a vortex mixer. An external magnet was used to separate the ferrofluid from the sample solution. The PAHs were extracted back into 500 μL of acetonitrile by vortex for 1 min and then using an external magnet to achieve phase separation. The eluate was transferred to a 1.5 mL centrifuge tube before being injected into the GC-FID for analysis.

GC-FID analysis

A Perkin Elmer Clarus500 gas chromatograph (Waltham, MA, USA) with a flame ionisation detector (FID) and a split/splitless injector was used to analyse the samples. The PAHs were separated using an Elite-5 fused silica capillary column (30 m×0.32 mm I.D, 1 μm film thickness) from J&W Scientific. The carrier gas was hydrogen at a 1.0 mL/min flow rate. The injection volume was set to 2 μL, and the sample injection mode was splitless. The GC oven temperature programme was set to begin at 70 °C (1 min), ramp to 170 °C at 7 °C/min, ramp to 290 °C at 15 °C/min, and hold at this temperature for 3 min. The injection port and detector were adjusted to 270 and 300 °C, respectively.

Box–Behnken design (BBD)

The extraction process was optimised using Box–Behnken design, and the statistical analysis of the experimental results was performed using Minitab® statistical software, version 21 (Minitab Inc., State College, PA, USA). Box BBD experiments were used to evaluate four independent parameters that could affect extraction efficiency, including the amount of MNPTEOS (5–15 mg), the volume of DES 1 (25–90 μL), salt addition (0–800 mg) and extraction time (1–10 min). To perform a quadratic regression on the model coefficients, 27 BBD experiment runs with four variables and three levels were utilised to develop a second-order polynomial model to describe the response variables and their interactions. The prediction equation was generated by fitting the experimental data to the BBD model in Eq. (1), which provides an empirical link between the response (total peak area) and the tested variables (coded level) at a confidence level greater than 95%:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (1)$$

where Y is the measure response (peak area), X_1 and X_2 are significant independent variables, β_1 and β_2 are the linear regression coefficients, β_{12} is the interactive regression coefficient and β_0 is a constant term. Further, to get maximum peak area, BBD was considered to analyse the effect of a significant independent variable on response.

The variables were coded as follows: X_1 (amount of MNPTEOS), X_2 (volume of DES 1), X_3 (addition of salt) and X_4 (extraction time). Table S-I of the Supplementary material to this paper contains the coded-level design variables and the predicted and experimental response values.

ANOVA, coefficient of determination (R^2), model significance (P-value of 0.05), non-significant lack of fit and response surface plots were used to validate the mathematical model generated by the BBD approach.

Analytical method validation and performance characteristics

The developed and optimised method has been validated employing SANTE guidelines for linearity, precision, limit of detection (*LOD*) and limit of quantification (*LOQ*).[31]

Real samples analysis

The wastewater samples were collected from the Kulim Hi-Tech industrial area in Kedah and Batu Kawan in Penang, Malaysia. The specimens were maintained at 4 °C under darkness until they were utilised. Prior to the LPME-BE procedure, the water samples were filtered with 0.45 μm membrane filters to remove contaminants. Roadside leaf samples continuously exposed to automobile air pollutants were randomly collected from various locations in Sungai Dua, Gelugor, Penang. The methodology employed for the preparation of leaf samples in this study was adapted from the previously reported procedure.[22] Initially, the leaves were minced

and subsequently pulverised using a mortar. Subsequently, roughly 0.5 g of leaves were subjected to introducing analytes at concentrations of 0.2, 0.5 and 1.0 μg mL^{-1}, followed by 15 min of diffusion. Subsequently, a volume of approximately 2 mL of acetonitrile was introduced into the mixture and agitated for 3 min using a vortex mixer. After centrifugation at 4000 rpm for 5 min, the supernatant was gathered and subjected to microextraction.

RESULTS AND DISCUSSION

Characterisation results

Fourier-Transform Infrared (FTIR) analysis. The confirmation of the presence of functional groups on the surface of the synthesised materials was conducted using FTIR analysis in the range of 400–4000 cm^{-1}. Fig. 1a–e displays the FTIR spectra of MNP, MNPTEOS and DES-based ferrofluids. Previous research has documented the FTIR spectra of various fatty acids and synthesised DES. Based on FTIR spectra in Fig. 1a and b, two prominent peaks associated with the Fe–O bond in MNP were seen at 577 and 611 cm^{-1}, respectively.[32,33] Furthermore, there are noticeable peaks in the O–H bending region at 3418 and 3422 cm^{-1} for MNP and MNPTEOS, respectively. MNPTEOS has a prominent peak at 1104 cm^{-1},

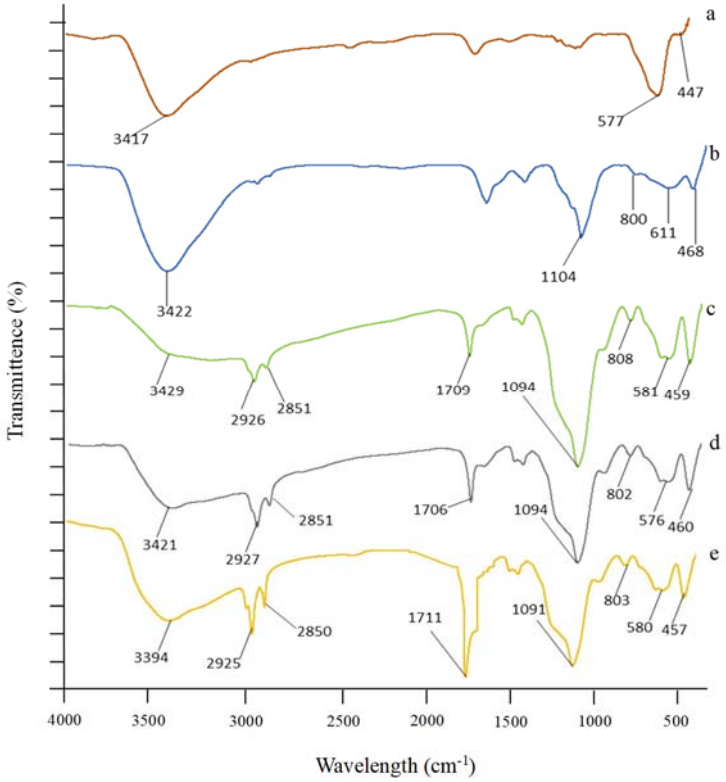

Fig. 1. FTIR spectra of (a) MNP, (b), MNPTEOS, (c) MNPDES 1, (d) MNPDES 2, (e) MNPDES 3.

representing the stretching of Si–O–Si bonds. This peak demonstrated the successful application of coating silanol groups on MNP cores.[33]

The FTIR spectra of the synthesised DES-based ferrofluids (MNPDES 1, 2 and 3) are displayed in Fig. 1c–e. The location of the O–H vibration peaks is affected by the analytes' structure and content. Carboxylic acids commonly form dimer associations due to hydrogen bonding.[34] The hydroxyl and carbonyl groups of DES-based ferrofluids are within the range of 3398 to 3407 cm^{-1} and 1705 to 1709 cm^{-1}, respectively. Characteristic peaks at 1094 (MNPDES 1), 1096 (MNPDES 2) and 1104 cm^{-1} (MNPDES 3) correspond to the Si–O–Si stretching vibration band, revealing the presence of TEOS in theses synthesised DES ferrofluids. Peaks at 2925 to 2927 cm^{-1} and 2851 to 2857 cm^{-1}, corresponding to the CH_3 and CH_2 groups, were found in all DES-based ferrofluids. This indicates that the DES successfully coated the MNP core.

Vibrating sample magnetometer (VSM) analysis. The magnetic properties of MNP, MNPTEOS and MNPDES were analysed at room temperature. The measurements were conducted within a magnetic field range of –8000 to 8000 G. The saturation magnetisation (*Ms*) and coercive field (*Hc*) were evaluated as critical magnetic properties. The magnetic properties predominantly dictate the behaviour of nanoparticles and ferrofluids. Fig. S-1a–e of the Supplementary material depicts the magnetic hysteresis of MNP, MNPTEOS and MNPDES, which have an S-shaped curve. *Ms* values for MNP and MNPTEOS were determined to be 55.86 and 35.67 emu g^{-1}, respectively. The magnetisation values for the synthesised MNPDES were measured to be 1.45, 1.51 and 1.50 emu g^{-1} for MNPDES 1, 2 and 3, respectively. The magnetic susceptibility of MNPTEOS and MNPDES is lower than that of the MNP. The presence of TEOS and DES on the surface of the MNP may lead to a decrease in the *Ms*, as predicted by Jayabharathi *et al.* [35] Furthermore, synthesising ferrofluid could contribute to the decrease in *Ms*.[36] Nevertheless, the decline in *Ms* seen in MNPDES suggests that the DES has successfully formed a coating on the surface of the magnetic nanoparticles (MNP). Despite having a lower *Ms* compared to MNP and MNPTEOS, MNPDES nevertheless fall within an acceptable range for simple separation using an external magnetic field.[35] The MNP, MNPTEOS and MNPDES exhibit superparamagnetism at room temperature, indicated by the lack of hysteresis, retentivity (*Mr*) and *Hc*.

Scanning electron microscopy – energy dispersive X-ray (SEM-EDX) analysis. The surface morphology of MNP, MNPTEOS and MNPDES was examined using SEM-EDX analysis. Fig. S-2a–e of the Supplementary material illustrates that MNP, MNPTEOS and MNPDES have a roughly spherical shape with a uniform distribution spanning 553.8 to 1.468 μm. The SEM analysis revealed that the synthesised MNPDES exhibited particle sizes ranging from 1.13 to 1.468 μm, greater than those of MNP (553.8 nm) and MNPTEOS (767.9 nm). The enlarged particle size in MNPDES is caused by the coating of the MNP core with DES.

Lenin *et al.* [37] observed that the application of DES coating on the surface of MNP's leads to an increase in the particle size of ferrofluids.

EDX analysis assessed the elemental composition of MNP, MNPTEOS and DES-based ferrofluids. The EDX spectra in Fig. S-3a of the Supplementary material indicate that the MNP is composed of 61.49 % Fe and 30.37 % O. MNPTEOS (Fig. S-3b) comprises 35.73 % Fe, 39.35 % O and 20.04 % Si. In the case of MNPDES (as shown in Fig. S-3c–e), all of them had a composition consisting of 32.41–36.96 % Fe, 37.07–39.20 % O and 15.50–21.57 % Si. Furthermore, MNPDES 3 contains the highest carbon content (13.04 %) compared to other compounds. This is attributed to a combination of lauric acid and capric acid in a mole ratio of 1:3, which results in the longest alkyl chain. The SEM-EDX results confirm the successful application of the DES coating on the surface of the MNPs.

Selection of fatty acid-based DES

The selection of an ideal DES as a carrier of ferrofluid is important in the proposed method. DES should have an appropriate interaction with the MNP to form a stable ferrofluid, thus enhancing the extraction efficiency of the developed method. Therefore, a hydrophobic DES should stabilise in an aqueous solution during microextraction. Moreover, the solubility of desired analytes in the extractant solvent, assisted with the presence of steric interaction, should be considered to achieve an optimum extraction efficiency. For these reasons, caprylic acid (C_8), pelargonic acid (C_9) and capric acid (C_{10}) acted as hydrogen bond donor (HBD) and were used to produce nine different eutectic solvents with lauric acid (C_{12}) by varying their molar ratio to investigate their extraction performance. The DES consist of caprylic acid, pelargonic acid, capric acid, and lauric acid incurporated with MNPTEOS, were listed in Table I.

TABLE I. Types of DES and their mole ratio; HBA: lauric acid

HBD	HBA:HBD mole ratio	Abbreviation
Caprylic acid	1:3	MNPDES 1
Pelargonic acid	1:3	MNPDES 2
Capric acid	1:3	MNPDES 3
Caprylic acid	1:2	MNPDES 4
Caprylic acid	1:4	MNPDES 5
Pelargonic acid	1:2	MNPDES 6
Pelargonic acid	1:4	MNPDES 7
Capric acid	1:2	MNPDES 8
Capric acid	1:4	MNPDES 9

Fig. 2 shows the effect of the type of DES on the LPME method. Generally, DES with longer alkyl chains and a high hydrogen bond donor (HBD) ratio, such as MNPDES 7 and 9, are expected to achieve higher recovery percentages for hydrophobic polycyclic aromatic hydrocarbons (PAHs), with the exception of

anthracene when using MNPDES 7. As illustrated in Fig. 2, both MNPDES 7 and 9 tend to self-coacervate and agglomerate in highly hydrophobic environments, which leads to a decrease in recovery percentages.[22] This behaviour suggests that MNPDES, containing longer alkyl chains and a higher HBD ratio, demonstrate increased viscosity. The elevated viscosity hinders the dispersion of MNP in the solution, thereby limiting the mass transfer of PAHs.[38]

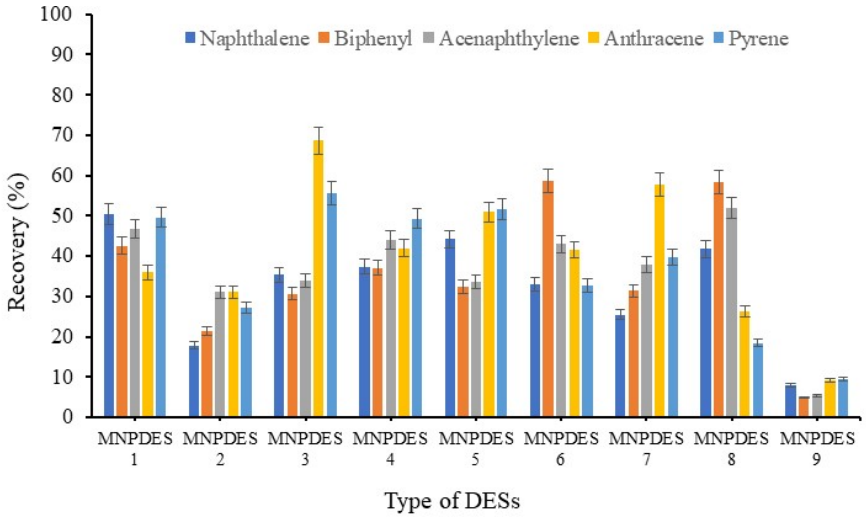

Fig. 2. The effect of type of DES with their mole ratio on extraction efficiency. Extraction condition: sample volume: 10 mL, amount of MNP: 10 mg, volume of DES: 50 µL, salt addition: 200 mg, extraction time: 5 min, back extraction solvent: ACN, volume of ACN: 300 µL, back-extraction time: 1 min.

Conversely, most PAHs displayed satisfactory recovery percentages when using DESs with shorter alkyl chains and a lower HBD ratio, specifically MNPDES 1, 4, 5 and 6. The results indicate that PAHs exhibit enhanced solubility in these MNPDESs, a phenomenon attributed to their lower viscosity and suitable hydrophobicity. This results in improved dispersion of MNPDES in the solution, facilitating mass transfer through stable hydrogen bonding and hydrophobic interactions between the PAHs and the alkyl chain of MNPDES 1. Additionally, the CH–π interaction between the benzene ring of PAHs and the CH_3 group of MNPDES also plays a crucial role in the efficient extraction process.[5,22] Several studies have highlighted the importance of these interactions between hydrophobic DES and analytes that contributed to more efficient extraction of non-polar compounds due to favourable dispersion interactions and phase compatibility.[4,5,22] Additionally, an increase in PAH solubility in DES formulated with hydrophobic DES, attributing this phenomenon to van der Waals and hydrophobic interact-

ions.[4,12,22] The DES, composed of long-chain components, effectively encapsulates non-polar analytes through hydrophobic inclusion or micelle-like domains, stabilizing them within the DES-rich phase. The observed selectivity and efficiency in PAH extraction can be linked to these interactions, even in the absence of strong polar interactions.[39,40]

As seen from Fig. 2, MNPDES 3 obtained a higher extraction efficiency on anthracene and pyrene, which are known as high molecular weight PAHs, owing to the optimal ratio of hydrogen bond donors and acceptors, which facilitated enhanced hydrophobic interactions with these compounds. Thus, the type and molar ratio of HBD are critical factors for effective extraction. After thorough analysis, MNPDES 1, consisting of a mixture of C_{12}:C_8 in a 1:3 ratio, has been identified as the most suitable carrier liquid for MNP. The extraction capability for all PAHs is enhanced due to its lower viscosity and satisfactory hydrophobic properties. The characteristics of DES 1 facilitate stable hydrogen bonding and hydrophobic interactions with PAHs, enabling efficient extraction from aqueous solutions.

Statistical analysis

The optimisation of independent parameters influencing the extraction efficiency was performed using BBD. The interaction and significance of each factor were determined by analysis of variance (ANOVA). The significance of each coefficient was determined using the *F*-test and p-value (Table S-II of the Supplementary material). The quadratic regression model indicated it was significant, as evidenced by the *F*-test with a low probability value (*p*-value of 0.05). The *F*-value of the model was 6.28, with a p-value of 0.001 (less than 0.05), implying a linear relationship between the amount of MNPTEOS (X_1), the volume of DES 1 (X_2), the addition of salt (X_3) and the extraction time (X_4). Notably, parameters such as X_1 and X_3 exhibited significant linear effects. Additionally, the quadratic term of X_2 and the interaction between X_2 and X_3 were also identified as significant. In contrast, X_4 and specific higher-order interactions did not reach statistical significance, indicating that their removal may enhance the model's simplicity.

The Lack-of-fit's *F*-value of 1.37 was observed insignificant (greater than 0.05) compared to the pure error. Furthermore, the reliability of the proposed model was assessed using R^2 and adjusted-R^2, which were 0.8799 and 0.7395, respectively, showing that the quadratic model is still suitable for data fitness and appropriate for the current study.[41,42] Despite low value of adjusted R^2, the model is statistically significant ($p = 0.001$) and generally exhibits a good fit. The lower adjusted R^2 suggests that not all predictors strongly influence the response variable.[43] As a result, the analysis demonstrates the significant importance of the model used to describe the relationship between the extraction conditions and the responses given, as shown in Eq. (2):

$$Y\,(\text{Total peak area}) = 16047 - 1251X_1 - 264X_2 + 36.2X_3 - 997X_4 + \\ + 79.5X_1^2 + 4.27X_2^2 - 0.00898X_3^2 + 53.3X_4^2 - 7.0X_1X_2 + \\ + 0.770X_1X_3 + 70.9X_1X_4 - 0.372X_2X_3 - 11.9X_2X_4 - 0.005X_3X_4 \quad (2)$$

The 3D response surfaces of the relationship between the amount of MNPTEOS, the volume of DES, the addition of salt, and the extraction time are shown in Fig. 3. According to Fig. 3a, adding 15 mg of MNPTEOS to a lower volume of DES 1 (25 μL) enhanced PAHs extraction efficiency. This behaviour indicates that the ferrofluid is more stable and provides higher MNP dispersion in the sample solution despite the low DES volume. Furthermore, Fig. 3b demonstrates that a high extraction efficiency of PAHs was obtained when 15 mg of MNPTEOS and 800 mg of NaCl were applied. Increasing the MNPTEOS provides more adsorption sites for PAHs. With the addition of salt, the solubility of PAHs in the aqueous phase decreases due to the salting out effect, thus increasing their extraction. The extraction efficiency of PAHs was low when a large volume of MNPDES 1 was used, and no salt was added (Fig. 3c). This is explained by the fact that increasing the volume of DES 1 increases the diluting impact of PAHs. Fig. 3d and e show that using a small amount of DES 1 and a large amount of MNP with a longer extraction period results in higher efficiency. Furthermore, as demonstrated in Fig. 3f, the extraction efficiency of PAHs increases with the addition of NaCl and the length of the extraction time. A higher concentration of NaCl and a longer extraction time may aid in developing equilibrium during the extraction process. It can be concluded that the overall results indicated the most optimal condition was using 15 mg of MNPTEOS, 25 μL of DES 1, 800 mg of NaCl and 10 min of extraction time.

It is crucial to acknowledge the variability in peak areas observed across experimental runs. These discrepancies can be attributed to sample handling and analytical drift factors. Minor variations in sample preparation, such as inconsistencies in vortex, pipetting, nanoparticle dispersion or magnetic separation, lead to fluctuations in analyte recovery, ultimately affecting chromatographic responses. Furthermore, analytical drift, which is characterized by gradual changes in instrument response over time, may stem from factors including temperature variations or detector instability. Such changes can compromise peak shape, baseline stability, and detector sensitivity.[44,45]

Analytical method validation and performance characteristics

The LPME-BE method for determining PAHs using DES-based ferrofluid was validated under optimum circumstances, as indicated in Table S-III of the Supplementary material. All PAHs demonstrated strong linearity between 0.005 and 5 μg mL^{-1}, with R^2 values larger than 0.9730. The detection and quantitation limits of detection (*LOD*) were determined to be 0.40–1.70 ng mL^{-1} and 1.33–5.67 ng mL^{-1}, respectively. The precision of the LPME-BE method was determined by

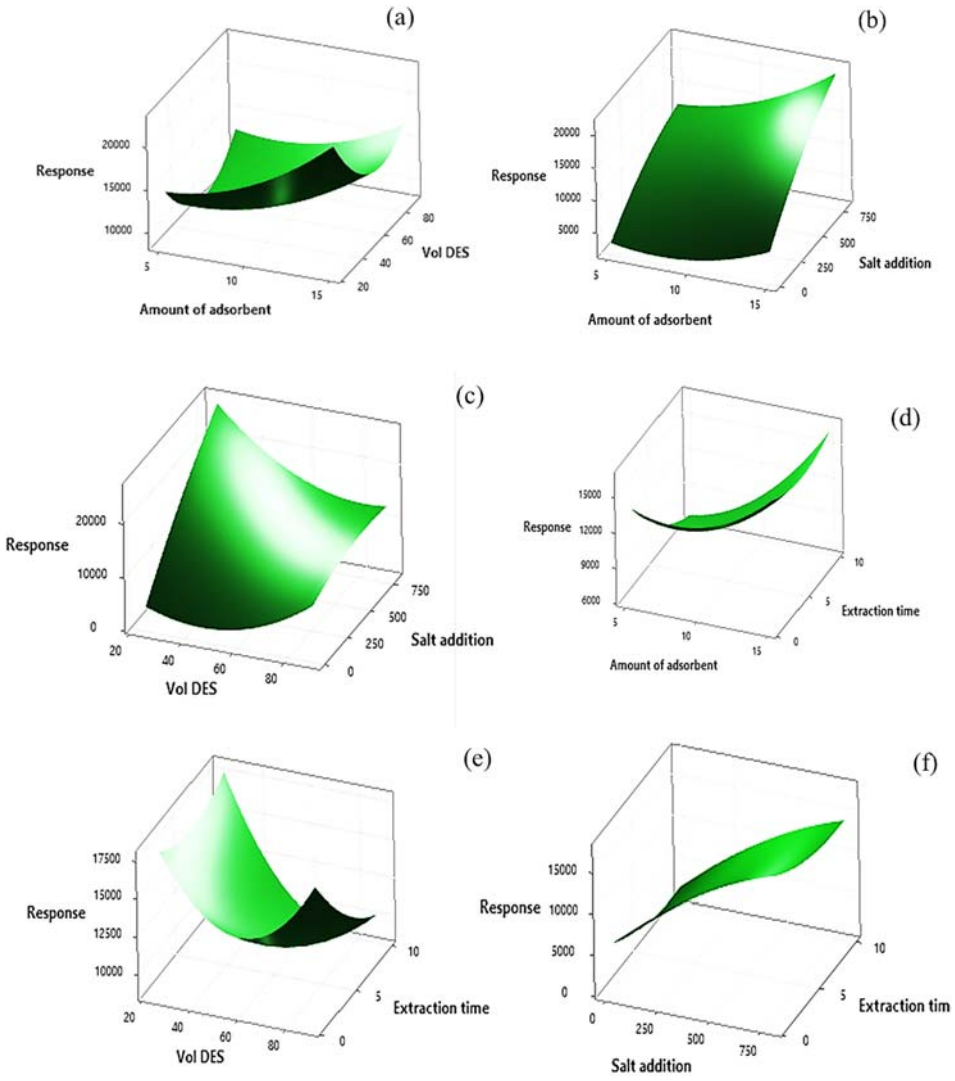

Fig. 3. Three-dimensional response surface plot in the peak area of PAHs. a) Amount of MNP-volume of DES. b) Amount of MNP-salt addition. c) Volume of DES-salt addition. d) Amount of MNP-extraction time. e) Volume of DES-extraction time. f) Salt addition-extraction time.

measuring the relative standard deviation (*RSD*) for intra-day (repeatability) and inter-day (reproducibility). The *RSD* ranges for intra-day (repeatability) and inter--day (reproducibility) precisions were 13.05–16.89 % and 9.37–15.84 %, respectively. It is notable that the intra- and inter-day precision values for the target PAHs ranged from 15–17 %, with recoveries as high as 118 %. According to SANTE

guidance document criteria, the *RSD* values should be fewer than 20 %, and the precision obtained with this approach is acceptable.[31] Considering the method demonstrated acceptable performance, however, increased variability in specific replicates might be contributed to minor inconsistencies in sample preparation. Additionally, analytical drift over time, particularly fluctuations in detector response and baseline shifts in GC-FID, may have contributed to signal variability across runs. These factors likely influenced the reproducibility of peak areas.[44,45] Although the precision values were generally acceptable for most analytes, these findings highlight the need for improved procedural consistency and the potential introduction of internal standards in future studies to enhance method robustness. Furthermore, the enrichment factor values for this method ranged from 26.66 to 40.05, indicating that the low concentration of PAHs was successfully preconcentrated utilising this extraction method.

Application to real samples

To evaluate the potential of the MNPDES, wastewater samples from industrial effluents (Batu Kawan, Seberang Perai, Penang and Kulim Hi-Tech industrial area, Kedah) and random roadside plant leaves samples, that were continuously exposed to air pollutants in automobile areas in Sungai Dua, Gelugor, Penang, were obtained and used to analyse the DES-based ferrofluid LPME-BE effectiveness by spiking PAHs into the samples with known amounts (0.2, 0.5 and 1.0 μg mL^{-1}). Table II shows the results of the extraction of PAHs for each spiked sample. No endogenous PAHs were detected in the wastewater and leaf samples; however, significant peaks appeared after adding exogenous PAHs. The method's extraction recoveries and repeatability were evaluated through triplicate analysis of samples spiked at concentration levels of 0.2, 0.5 and 1 μg mL^{-1} of each PAHs. The data presented in Table II indicate that the recoveries of PAHs from environmental samples fall within the range from 75.78 to 118.65 %, with relative standard deviations (*RSD*s) ranging from 0.08 to 13.21 %. As per the guidelines provided by SANTE, the outcomes of the recovery were deemed satisfactory.[31] The research findings demonstrated that the LPME-BE using MNPDES is a viable technique for efficiently separating, extracting, and quantifying trace amounts of PAHs in diverse, complex environmental matrices.

TABLE II. Determination of analytes using the proposed method in different real samples; ACN – acenaphthylene; Ww – wastewater; L – leaves

Sample	Spiked conc. μg/mL	Relative recovery, % (*RSD* / %), *N* = 3				
		Naphthalene	Biphenyl	ACN	Anthracene	Pyrene
Ww 1	0.2	98.68 (9.81)	79.26 (4.02)	85.08 (1.03)	105.45 (1.07)	80.91 (9.87)
	0.5	92.42 (1.01)	82.10 (7.29)	89.04 (7.72)	104.93 (0.47)	89.85 (1.21)
	1	92.05 (0.41)	82.14 (5.45)	86.14 (2.14)	109.40 (7.52)	89.14 (7.87)

TABLE II. Continued

Sample	Spiked conc. µg/mL	Relative recovery, % (*RSD* / %), *N* = 3				
		Naphthalene	Biphenyl	ACN	Anthracene	Pyrene
Ww 2	0.2	99.33 (5.23)	77.78 (1.41)	85.10 (0.03)	110.40 (8.66)	89.15 (0.02)
	0.5	95.60 (6.81)	97.23 (6.10)	82.56 (5.27)	106.25 (1.91)	89.31 (0.24)
	1	97.18 (2.73)	89.58 (13.21)	89.45 (4.26)	117.44 (0.42)	89.62 (0.93)
Ww 3	0.2	101.86 (9.05)	81.20 (7.31)	90.41 (10.21)	117.50 (0.51)	89.40 (0.48)
	0.5	93.21 (7.75)	84.78 (8.60)	85.30 (0.46)	117.56 (0.59)	93.18 (7.52)
	1	101.13 (9.40)	78.96 (4.29)	93.05 (9.95)	112.77 (5.49)	92.49 (6.27)
Ww 4	0.2	109.39 (11.64)	87.94 (11.20)	86.38 (2.61)	115.91 (1.86)	89.25 (0.11)
	0.5	102.85 (12.13)	81.14 (8.83)	97.99 (12.32)	117.15 (0.56)	89.27 (6.92)
	1	101.65 (10.22)	82.04 (5.66)	86.90 (1.91)	118.65 (2.19)	94.33 (6.62)
Ww 5	0.2	96.08 (0.77)	77.53 (2.26)	88.53 (6.74)	107.07 (9.91)	90.06 (0.92)
	0.5	94.95 (1.05)	83.23 (10.59)	85.46 (0.64)	113.55 (4.48)	99.06 (14.16)
	1	99.47 (6.65)	75.78 (1.63)	85.28 (0.40)	110.60 (10.27)	94.23 (9.36)
Ww 6	0.2	95.07 (0.86)	76.30 (1.30)	76.48 (0.95)	114.58 (6.89)	90.57 (2.73)
	0.5	99.50 (6.66)	76.47 (0.61)	85.41 (0.68)	108.92 (6.80)	89.50 (0.70)
	1	96.06 (0.61)	77.66 (1.20)	87.11 (3.30)	118.10 (1.12)	89.56 (0.66)
Ww 7	0.2	101.78 (8.52)	77.38 (0.70)	85.45 (0.62)	117.29 (0.16)	89.26 (0.20)
	0.5	96.47 (1.20)	75.94 (5.09)	86.04 (1.57)	118.65 (1.78)	89.15 (0.92)
	1	95.59 (0.08)	82.37 (9.94)	86.21 (2.0)	116.96 (0.23)	89.47 (0.53)
L 1	0.2	95.72 (0.11)	77.61 (1.10)	85.01 (1.02)	117.10 (0.72)	88.90 (1.92)
	0.5	96.20 (0.80)	77.38 (0.70)	85.00 (1.20)	114.93 (2.74)	89.54 (0.63)
	1	96.35 (1.03)	76.00 (1.88)	81.83 (5.60)	110.88 (8.00)	88.74 (0.64)
L 2	0.2	94.72 (1.38)	76.20 (1.50)	84.71 (0.61)	113.01 (5.18)	88.25 (1.43)
	0.5	95.31 (0.50)	71.24 (11.44)	84.70 (0.62)	114.78 (2.92)	85.83 (5.45)
	1	90.88 (7.41)	71.07 (11.80)	77.88 (13.07	112.27 (6.15)	88.04 (1.76)
L 3	0.2	89.10 (10.38)	75.06 (3.64)	82.20 (4.96)	113.53 (4.51)	86.44 (4.40)
	0.5	95.15 (0.73)	76.56 (0.82)	80.98 (7.15)	112.52 (5.82)	88.58 (0.90)
	1	94.83 (1.22)	77.2 (0.624)	87.28 (3.57)	115.61 (1.99)	85.22 (6.95)

CONCLUSION

The proposed work showed that the application of the Box–Behnken design optimized independent parameters impacting the DES-based ferrofluid LPME-BE method and achieved appropriate PAHs determination in environmental samples. The primary goal of this work is to determine the maximum peak area by evaluating significant independent parameters that affect the PAHs peak area. The successful DES coating on MNP has been conclusively validated through FTIR, VSM and SEM-EDX analyses. The ANOVA test indicated that these parameters substantially impacted the extraction efficacy of PAHs using DES-based ferrofluids. The optimum extraction performance within the experimental range was expected to be 15 mg of MNPTEOS, 25 µL of DES 1, 800 mg of NaCl and 10 min of extraction time. Validation of analytical performances such as precision, accuracy, enrichment factor and recovery were evaluated under optimal extraction circum-

stances. The proposed extraction method is suitable for measuring PAHs in environmental samples. The *LOD* and *LOQ* values for PAHs were 0.4–1.70 ng mL^{-1} and 1.33– 5.67 ng mL^{-1}, respectively, which made the approach dependable for reliably and precisely determining PAHs. The recoveries obtained were ranged from 75.78 to 118.65 % with *RSD* < 15 %. As a result, it has been demonstrated that the established approach is straightforward and reliable for quantifying PAHs in environmental samples.

This study introduces a promising green extraction method for the determination of PAHs that utilises DES-based ferrofluids. However, it is important to recognise certain limitations. The method was assessed with a limited selection of PAHs and examined solely in spiked matrices, potentially failing to exhibit the actual environmental sample applications. Intra- and inter-day precision variability may result from slight inconsistencies in sample handling and analytical drift. The application of GC-FID, although practical, is insufficient in selectivity for dependable analyte identification within complex matrices. The statistical model demonstrated moderate explanatory power and variability in response despite its utility. Future research should enhance the method's applicability by incorporating validation with certified reference materials (CRMs) and real contaminated samples, integrating internal standards, assessing matrix effects *via* spiking or standard addition and confirming results through mass spectrometry (GC–MS or LC–MS/MS) to achieve greater selectivity. The proposed enhancements will increase the method's robustness, reliability and relevance for trace-level environmental analysis.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13221, or from the corresponding author on request.

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ИЗВОД

ОПТИМИЗАЦИЈА ТЕЧНЕ МИКРОЕКСТРАКЦИЈЕ РАН ЗАСНОВАНЕ НА ДУБОКОМ ЕУТЕКТИЧКОМ РАСТВАРАЧУ У УЗОРЦИМА ЖИВОТНЕ СРЕДИНЕ КОРИШЋЕЊЕМ МЕТОДОЛОГИЈЕ ПОВРШИНЕ ОДЗИВА

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Присуство полицикличних ароматичних угљоводоника (PAHs) у отпадним водама представља значајан ризик по здравље. У циљу решавања овог проблема, развијен је нови ферофлуид на бази дубоког еутектичког растварача (DES-ферофлуид) за микроекстракцију у течном фазном стању са повратном екстракцијом (LPME-BE) ради детекције PAHs.

DES-ферофлуид је карактерисан у погледу својих физичко–хемијских особина и морфологије помоћу FTIR, VSM и SEM-EDX. Кључни параметри у процесу микроекстракције у течном фазном стању са повратном екстракцијом (LPME-BE) оптимизовани су применом методологије површине одзива (RSM), засноване на Box–Behnken дизајну (BBD). Оптимални услови укључивали су 15 mg магнетних наночестица премазаних тетраетилортосиликатом (MNPTEOS), 25 µL DES 1 (мешавина каприлне и лауринске киселине), 800 mg NaCl и време екстракције од 10 min. Анализа варијансе (ANOVA) је потврдила добро поклапање експерименталних података са моделом, са R^2 од 0,8799 и прилагођеним R^2 од 0,7395. Метод је постигао границу детекције (*LOD*) у опсегу од 0,4 до 1,7 ng mL^{-1} и границу квантификације (*LOQ*) од 1,33 до 5,67 ng mL^{-1}. Приноси за узорке са додатим стандардом кретали су се од 75,78 до 118,65 %, са релативним стандардним одступањем (*RSD*) мањим од 15 %. Овај LPME-BE метод са DES-ферофлуидом и BBD оптимизацијом представља обећавајућу и ефикасну алтернативу за детекцију PAHs у узорцима отпадних вода.

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