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Article

A Novel Protocol to Monitor Trace Levels of Selected Polycyclic Aromatic Hydrocarbons in Environmental Water Using Fabric Phase Sorptive Extraction Followed by High Performance Liquid Chromatography-Fluorescence Detection

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Abstract: Fabric phase sorptive extraction (FPSE) combines the advanced material properties of sol–gel derived microextraction sorbents and the flexible and permeable fabric support to create a robust, simple and green sample preparation device. It simultaneously improves the extraction sensitivity, and the speed of the extraction by incorporating high volume of sponge-like porous sol–gel hybrid inorganic–organic sorbents into permeable fabric substrates that is capable of extracting target analytes directly from simple to complex aqueous sample matrices. For the first time, this technique was applied to the trace level determination of selected polycyclic aromatic hydrocarbons (PAHs) in environmental water samples using a non-polar sol–gel C18 coated FPSE media. Several extraction parameters were optimized to improve extraction efficiency and to achieve high detection sensitivity. Validation tests of spiked samples showed good linearity for four selected PAHs (R^2 = 0.9983–0.9997) over a wide range of concentrations (0.010-10 ng/mL). Limits of detection (LODs) and quantification (LOQs) were measured at pg/mL levels, 0.1–1 pg/mL and 0.3–3 pg/mL, respectively. Inter- and intra-day precision tests showed variations of 1.1–4.1% for four selected PAHs. Average absolute recovery values were in the range of 88.1–90.5% surpassed the recovery prediction model, with relative standard deviations below 5%. The developed FPSE-HPLC-FLD protocol was finally applied to analyze 8 environmental water samples. Out of four selected PAHs, fluoranthene (Flu) and phenanthrene (Phen) were the most frequently detected in four samples, at concentration levels of 5.6–7.7 ng/mL and 4.1-11 ng/mL, respectively followed by anthracene (Anth) and pyrene (Pyr) in two samples. The newly developed FPSE-HPLC-FLD protocol is simple, green, fast and economical, with adequate sensitivity for trace levels of four selected PAHs and seems to be promising in routine monitoring of water quality and safety.

Keywords: Fabric Phase Sorptive Extraction (FPSE); Polycyclic Aromatic Hydrocarbons (PAHs); Persistent pollutants; Green Analytical Chemistry (GAC); Environmental water; Sorptive microextraction

1. Introduction

Polycyclic aromatic hydrocarbons are toxic, carcinogenic and mutagenic organic compounds, possessing immunologic and reproductive effects. They are introduced into the environment from both natural and anthropogenic sources, typically formed as unintentional byproducts of the

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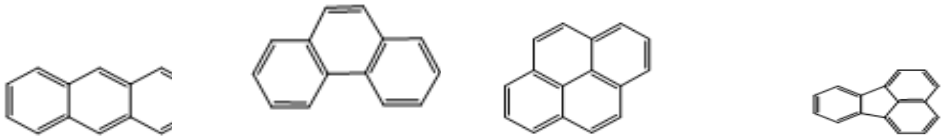
incomplete combustion of organic matter. PAHs are ubiquitous in nature and the most damaging pollutants with regard to the ecosystem **[1-5]**. Although they have low water solubility and exist in environment at very low concentrations, PAHs are strongly bioaccumulative (e.g. by fish) and can thus pass up the food chain to top predators, including humans **[6-7].** Natural inputs such as forest and prairie fires, volcanic eruptions and anthropogenic inputs such as oil spills, waste incineration, coke and asphalt production, oil refining, aluminum production, urban runoff, emission from combustion and industrial processes are the main sources of PAHs in the environment **[8-10]**. The elevated concentrations of PAHs in environment, together with their ecological toxicity and health risk for humans, have spawned their numerous environmental studies **[11-14]**. Due to their environmental concern, PAHs are included in the USEPA (United States Environmental Protection Agency) and in the EU (European Union) priority lists of pollutants **[15].** Human exposure to PAHs occurs mainly by direct inhalation of polluted air and tobacco smoke, dietary intake of smoked food stuffs, direct intake and contact with contaminated water, direct contact with contaminated soil and dermal contact with soot, tars and oils **[16]**. PAHs enter the aquatic environment through run off from contaminated roads or sealed parking lots **[17]**, urban and industrial waste water discharge, direct spillage, wet and dry deposition of atmospheric born contaminants **[18]**. Therefore, trace determination of PAHs in water matrices **[19]** is considered to be a valuable tool in risk assessment, remediation and management of PAHs in the environment.

In this study, four selected small PAHS including anthracene (a linear three ring PAH), phenanthrene (a fused three ring PAH), pyrene (a fused four ring PAH), and fluoranthene (a fused four ring PAH), and fluoranthene (a non-alternant four ring PAH) (Figure 1).



A B C D

Figure 1. Chemical structures of four selected PAHs: (A) Anthracene; (B) Phenanthrene; (C) Pyrene; (D) Fluoranthene.

Anthracene is used as a raw material, in the industrial production of hydrogen peroxide and in the manufacturing of alizarin and anthraquinone dyes for cotton fibers **[20]**. Phenanthrene, a tricyclic aromatic hydrocarbon, has a "K-region and a "bay-region", where the main carcinogenic species can be formed and is therefore commonly used as a model substrate for carcinogenic studies **[21].** Fluoranthene, a non-alternant PAH, contains a five-member ring, and is an environmental persistent organic pollutant, structurally similar to other environmentally important compounds such as fluorene, dibenzothiophene, acenaphthylene, carbazole, dibenzofuran, and dibenzodioxin, i.e. compounds of **[22-23].** Pyrene, occurs at relatively high concentrations in PAH mixtures and one of the most highly concentrated PAH detected in drinking water **[24]**.

Until now, numerous protocols comprising gas chromatography (GC) and high performance liquid chromatography (HPLC) have been successfully developed for the determination of PAHs in the environment **[25-29]**. However, since PAHs are generally present at trace levels in the environment and are accompanied by diversified matrices, they cannot be directly handled by analytical instruments. Thus sample pretreatment aims to concentrate the PAHs as well as to eliminate or decrease the interference, is inevitable **[30]**. For the isolation of PAHs in

environmental matrices, various sample pre-treatment techniques have been proposed including solid-phase extraction (SPE) **[31-32]**, liquid-liquid extraction (LLE) **[33]**, miniaturized homogeneous liquid-liquid extraction (MHLLE) **[34],** solid-phase microextraction (SPME) [**35-36]** and single drop microextraction (SDME) **[37-38]**. Although these techniques are very useful but possess some serious drawbacks including laborious, time consuming, high consumption of organic solvents and sample solutions, and often lead towards significant loss of analytes and poor reproducibility (LLE, SPE), high cost, fragility of the fiber, low thermal and chemical stability, swelling if exposed to organic solvents, limited lifetime and sample carry-over (SPME) **[39-40],** breaking of the organic drop due to fast stirring, reduced extraction rate due to air bubble formation, time-consumption and non-equilibrium in most cases (SDME) **[41].**

Nowadays, a number of unique materials have been synthesized and applied as sorbents for PAH extraction, including multi-walled carbon nanotubes **[32]**, magnetic nanoparticles **[42]**, and metal–organic frameworks **[43]**. However, the synthesis process of such materials is rather complex and often consumes large amounts of organic solvents and time. Therefore, new, versatile and high-performance adsorbents with a simple preparation process, are still highly desirable.

Kabir et.al, in 2014 integrates the rich surface chemistry of cellulose cotton fabric substrates and sol–gel technology, to develop a novel sorptive microextraction technique called fabric phase sorptive extraction (FPSE) The inherent advantages of the synthesized sorptive material include (1) flexible hydrophobic/hydrophilic fabric substrate that can be bent, twisted and squeezed to insert directly into unmodified samples; (2) ability to extract target analytes directly from raw sample matrix i.e. environmental water, whole milk, whole blood, urine, saliva, containing particulates, biomasses, debris without any sample pre-treatment; (3) high loading capacity, unique and tunable selectivity; (4) any organic or aqueous-organic solvent mixture can be used for elution/ solvent back-extraction; (5) capable of extraction of polar, nonpolar, acidic, and basic compounds; (6) no solvent evaporation and analyte reconstitution is needed; (7) operational simplicity, meets green analytical chemistry and economic criteria as well. One major advantage of FPSE is its ability to immobilize highly polar polymers in a sol–gel hybrid organic–inorganic network, chemically bonded to the fabric substrate, resulting in a microextraction material that can efficiently extract both polar and nonpolar analytes directly from aqueous sample matrix and has been used in the analysis of a wide variety of analytes in environmental and biological samples **[44-47]**.

As PAHs are inherently nonpolar, hydrophobic compounds which do not ionize in water. Intuitively, the selective extraction of highly nonpolar analytes e.g. PAHs from aqueous sample matrix would be highly facilitated with a hydrophobic sorbent as the extraction phase material **[48]** and consequently sol-gel C18 coated FPSE media containing long hydrophobic C18 chains has been evaluated and successfully applied in this case.

Herein, we describe the design and preparation of sol–gel C18 coated FPSE media and the development of a novel application protocol for the efficient extraction of trace amounts of four selected small PAHs of high environmental interest from aqueous solutions. The hydrophilic cellulose fabric substrate incorporated in the core of the adsorbent aids in the extraction kinetics by attracting water molecules containing PAHs towards its surface for a successful sorbent-analyte interaction, resulting in trapping of the analyte on FPSE media. Our strategy offers the following advantages in water analysis: (1) highly efficient analysis of PAHs (at the ppt level concentrations); (2) simultaneous determination of four different PAHs in water using just one adsorbent; (3) facile regeneration of the adsorbent; (4) no solvent evaporation and analyte reconstitution is needed (5) the FPSE strategy can be extended to design a range of adsorbents for sensitive determination of related pollutant compounds. This study serves the analytical and environmental community by providing a better and superior pathway for effective extraction and determination of trace PAHs in water matrices of domestic and environmental interest. In addition, HPLC-FLD was used for chromatographic separation of four PAHs with advantage of a shorter analytical time, superior

resolution and sensitivity. Finally and most importantly, this is the first manuscript presenting FPSE application for the trace analysis of polycyclic aromatic hydrocarbons in aqueous media.

2. Materials and Methods

2.1. Choice of target PAHs

The selected four small, low aromatic PAHs are in the list of 16 priority PAHs designated by USEPA **[1]** and are either source markers in water i.e. diagnostic ratios for discriminating petrogenic from pyrolytic sources (fluoranthene/pyrene) **[27]**, model substrate for cancer studies (phenanthrene) **[21],** and the PAHs detected in the highest concentrations in drinking water (fluoranthene, phenanthrene, pyrene, and anthracene) **[49].** Besides, feasibility of HPLC-FLD analysis, availability of economic PAHs analytical standards is also considered.

2.2 Chemicals and materials

Substrates for sol-gel C18 coated FPSE media, Muslin 100% cotton cellulose were purchased from Jo-Ann Fabric (Miami, FL, USA). Precursors for sol-gel synthesis, octadecyl trimethoxysilane, tetramethyl orthosilicate, trifluoroacetic acid (TFA), organic solvents such as acetone and dichloromethane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide and hydrochloric acid were purchased from Thermo Fisher Scientific (Milwaukee, WI, USA).

Certified analytical standards of anthracene (Anth), fluoranthene (Flu), pyrene (Pyr), and phenanthrene (Phen) were purchased from Sigma-Aldrich (Darmstadt, Germany). HPLC grade methanol, acetonitrile and water were purchased from Sigma-Aldrich (Darmstadt, Germany) and filtered through 0.22 μm filter before use. Stock solution of each PAH at a concentration of 1 mg/mL, and a standard mixture of all four PAHs (1 mg/ml each) were prepared in methanol to achieve a response at the comparable level in HPLC-FLD. Working solutions were freshly prepared by diluting the mixed standard solution with HPLC grade water to the required concentrations. All standards and working solutions were stored at 4°C. All other reagents were of analytical grade.

2.3 Instrumentation

HPLC-FLD analysis of four PAHs was performed with a Dionex P680 HPLC pump (Germany) equipped with a Dionex Ultimate 3000 Fluorescence detector and a Chromeleon chromatography management software (Dionex, Germany). An Acentis Express reverse phase C18 column (10 cm × 4.6 mm, particle size 5 μm, Supelco, Germany) was used for separation. Ultrasonic degassing was performed with ultrasonic bath (Sarthak Scientific Services, Panchkula, India).

2.4 Water sample collection

The water samples selected for the investigation included two river water samples collected from the Chakki river (Pathankot, Punjab, India), two rain water samples and two bore well drinking water samples collected from the Punjabi university campus, (Patiala, Punjab, India), two metal factory wastewater collected from a chemical factory (Patiala, India). Before the experiments, all the water samples were filtered through Whatman filter paper and then through 0.22 μm micropore membranes and stored at 4 °C in a refrigerator.

2.5 Preparation of sol-gel C18 coated FPSE media

Taking the nonpolar, hydrophobic characteristics of the selected PAHs into consideration (log K_{ow} values between 4.45 and 5.16), it is obvious that a nonpolar sorbent would provide the best affinity towards the PAHs to selectively isolate them from complex environmental water sample matrix. As such, octadecyl trimethoxysilane was selected as the source of C18 pendant group in the sol-gel silica network during the sol solution design. C18 has long been known as a nonpolar sorbent

in solid phase extraction as well as a nonpolar stationary phase in reversed phase liquid chromatography. It is worthy to mention that the selectivity of commercially available C18 sorbent is substantially different from that of sol-gel C18 sorbent and often the later demonstrates unique selectivity towards wide range of analytes including polar, medium polar and nonpolar analytes due the presence of residual surface silanol groups in the sol-gel C18 sorbent matrix.

Unlike substrate used in C18 sorbent (silica particles), the substrate used in fabric phase sorptive extraction plays an active role in determining the overall polarity and selectivity of the FPSE media. A fabric substrate made up of 100% cotton cellulose was selected as the support for sol-gel C18 coating so that the hydrophilic nature of the substrate may persuade water molecules to come close to the extraction device during extraction in order for the requisite interactions between C18 pendant groups and the PAHs, resulting in successful extraction of PAHs onto the FPSE media. The permeable structure of the fabric support also plays important roles as a pseudo solid phase extraction disk and facilitate to the rapid analyte extraction due to the continuous diffusion of water through the FPSE media under the influence of magnetic stirring during the extraction.

Due to the presence of starch and other finishing chemicals on the cellulose fabric substrate, the substrate requires a thorough cleaning. In the same time, a chemical treatment to the substrate that maximize the number of available hydroxyl groups required to effectively bind sol-gel network via condensation reaction was applied. Preliminary cleaning of the substrate was accomplished by immersing a 100 cm^2 section of cotton cellulose fabric in deionized water for 30 min under constant sonication followed by multiple rinsing with profuse amount of deionized (DI) water. For the surface chemical activation, the fabric was immersed in 1M NaOH solution for an hour under constant sonication. This step was followed by thoroughly rinsing with DI water and treating with 0.1 M HCl solution for an hour to neutralize residual NaOH which might still be on the fabric surface. Finally, the cleaned and activated cellulose substrate was dried and stored in an air-tight container until it is used for sol-gel C18 coating.

Sol solution to create sol-gel C18 coated FPSE media was prepared by sequentially mixing sol-gel precursor, methyl trimethoxysilane (MTMS); solvents methylene chloride and acetone; organically modified sol-gel precursor, octadecyl trimethoxysilane; sol-gel catalyst, trifluoroacetic acid (TFA); and water. In order to obtain uniform sol solution for fabric substrate coating, the molar ratio between methyl trimethoxysilane precursor: methylene chloride: acetone: trifluoroacetic acid: water was maintained at 1: 2.33: 1.94: 0.5: 0.20. The molar ratio between methyl trimethoxysilane and octadecyl trimethoxysilane were maintained at 1: 0.38. To ensure that all the sol solution ingredients mix up homogeneously, the sol solution was vigorously vortexed for 3 min after adding each of the ingredients. The final sol solution was centrifuged for 5 min, followed by collecting the supernatant into a clean 3 oz. amber color glass reaction bottle. The solution was then sonicated for 10 min to remove trapped gaseous molecules from the sol solution.

The cleaned and pretreated cellulose fabric substrate was then gently immersed into the sol solution to initiate the substrate coating *via* dip coating process. The substrates were kept into the appropriate sol solutions for 2 hrs. During this surface coating period, a three dimensional sol-gel network chemically bonded to the substrate was formed. At the end of the coating period, the sol solutions were expelled from the reaction bottle, the coated fabrics were dried in a desiccator and finally the sol-gel sorbent coated FPSE media were conditioned/aged in a home-made thermal conditioning device built inside a gas chromatography oven with continuous helium gas flow at 50°C for 24 h. At the end of conditioning/ageing, sol-gel C18 coated FPSE media were cleaned sequentially with CH_2Cl_2 and CH_3OH. Finally, the FPSE media were dried in presence of continuous helium gas flow at 50°C for 1 h. The FPSE media were then cut into 2.5 cm x 2.0 cm piece, typical application size for FPSE and stored in an airtight glass container for future use.

2.6 Fabric phase sorptive extraction procedure

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Firstly, the sol-gel C18 coated FPSE media was conditioned in a mixture of 1 mL CH_3OH and 1 mL ACN for 5 min and then rinsed with 2 mL deionized water to remove residual organic solvents. The FPSE procedure is shown in Figure 2.

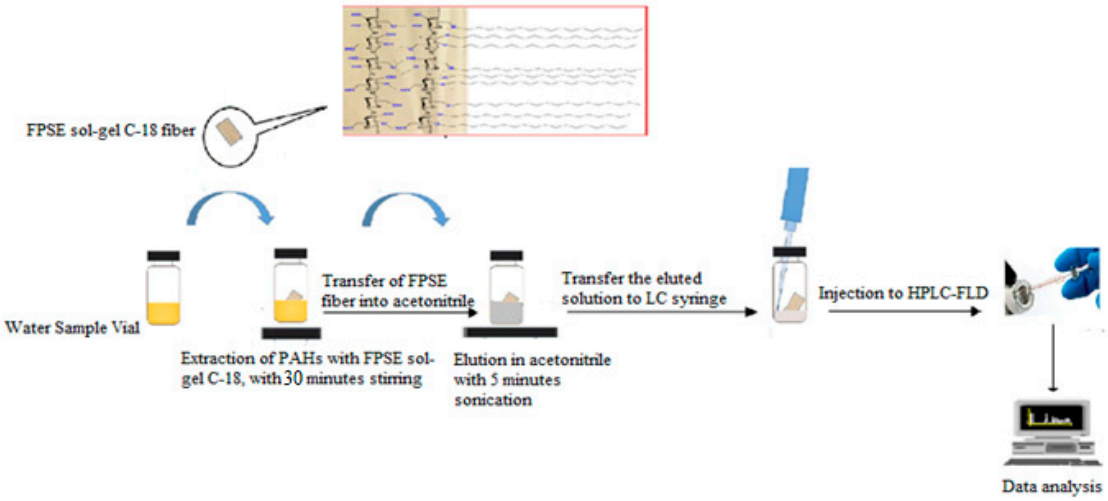

Figure 2. Chemical structure of sol-gel C18 coated FPSE media and the schematic representation of FPSE protocol.

Briefly, the extraction process was performed in a 20-mL glass vial containing 10 mL of sample aqueous solution. A sol-gel C18 coated FPSE media was directly immersed in the sample solution for 30 minutes under constant stirring at 1000 rpm at room temperature. After extraction, the extracted PAH were eluted from the sol-gel C18 coated FPSE media in 300 µL of acetonitrile with sonication for 5 minutes. Finally, 20 µL of this solution was injected directly into the HPLC-FLD system for the analysis.

2.7 HPLC-FLD analysis

Determination of PAH was carried out on a HPLC-FLD system. Chromatographic analysis was performed using an Acentis Express C18 column maintained at 25 °C. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B) and the flow rate was 1 mL/min. The isocratic elution mobile phase was set as follows: 15% A and 85% B with a total chromatographic run time of 10 min at λ_{ex}= 260 nm and λ_{em}= 420 nm using a fluorescent detector. The injection volume was 20 µL.

3. Results and discussion

3.1. Characterization of sol-gel C18 coated FPSE media

3.1.1. Scanning Electron Microscopy

Figure 3 represents the scanning electron micrographs (SEM) of (a) uncoated Muslin cotton (100% cellulose) substrate at 100x magnification; (b) sol-gel C18 coated fabric phase sorptive extraction media at 100x magnification; and (c) enlarged image of sol-gel C18 coated fabric phase sorptive extraction media at 500x magnification.

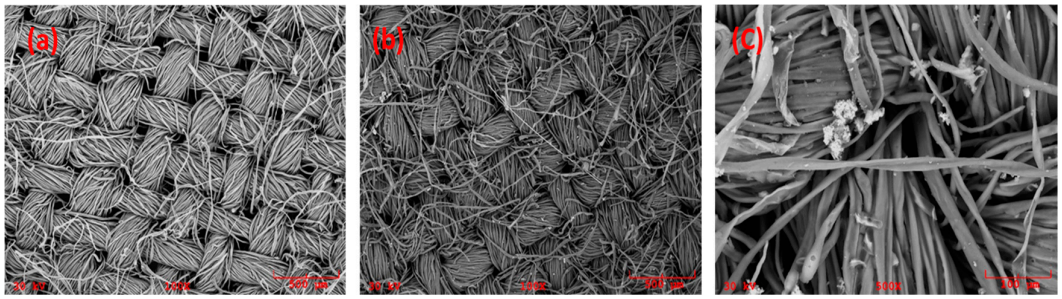

Figure 3. Scanning electron microscopy images of (a) uncoated cotton (100%) cellulose fabric substrate at 100x magnification; (b) uniformly coated sol–gel C18 sorbent coating on fabric matrix at 100x magnification; (c) enlarged image of sol-gel C18 coated FPSE media at 500x magnification.

Since the mechanism of analyte extraction by fabric phase sorptive extraction largely depends on the sponge-like porous architecture of sol-gel sorbent coating (for faster analyte diffusion) as well as the permeability of the substrate (should be retained even after the sorbent coating) that mimics a solid phase extraction disk to allow aqueous sample matrix to flow through it, resulting in intimate analyte-sorbent interaction, followed by successful extraction, it is important to study the surface morphology of the FPSE media before and after the sorbent coating. The SEM images demonstrate the microstructures and the well preserved through pores of the cellulose substrate even after the sol-gel C18 sorbent coating. This flow-through extraction mechanism is only exploited in solid phase extraction (SPE), and is totally absent in solid phase microextraction and related techniques (such as stir bar sorptive extraction, thin film microextraction, etc.) due to the impermeable nature of the substrate used in these microextraction techniques. The flow-through extraction system consequently helps in achieving faster extraction equilibrium. The enlarged image of the sol-gel C18 coated FPSE media demonstrates that C18 coatings are homogeneously distributed on the fabric substrate surface while maintaining the through pores of the substrate.

3.1.2. Fourier-Transform Infrared Spectroscopy (FT-IR)

Figure 4 illustrates FT-IR spectra representing (a) uncoated Muslin cotton (100% cellulose) fabric; (b) octadecyl trimethoxysilane; (c) sol-gel C18 coated fabric phase sorptive extraction media.

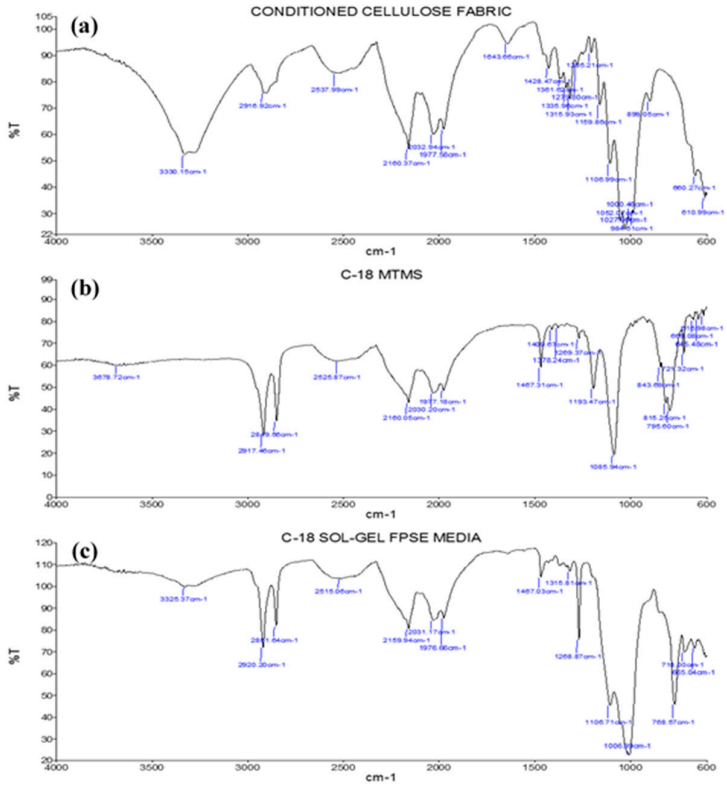

Figure 4. FT-IR spectra of (a) uncoated cellulose substrate; (b) octadecyl trimethoxysilane; and (c) sol–gel C18 coated FPSE medium.

The FT-IR spectra of the uncoated Muslin cotton 100% cellulose fabric demonstrates characteristics absorption bands at ~3330 cm^{-1}, 2916 cm^{-1}, 1315 cm^{-1}, and 1028 cm^{-1} that correspond to O-H, C-H, C-O vibration and C-H bending vibration, respectively **[50]**. The characteristic peaks of octadecyl trimethoxysilane appear at 2917 cm^{-1}, and 2849 cm^{-1} which correspond to asymmetric and symmetric vibrations of $–CH_2$-, -CH_2- groups, respectively **[51]**. The strong peak at 1467 cm^{-1} and 1085 cm^{-1} are due to the vibration absorption of Si-O-C, and Si-O-Si, respectively. Besides, the strong peak at 815 cm^{-1} is assigned to Si-C bond and that at 795 cm^{-1} is assigned to the vibration of $(\text{-}CH_2\text{-})_n$ ($n \geq 4$) **[52].** The characteristic peaks of sol-gel C18 coated FPSE media appeared at 2890 cm^{-1} and 2851 cm^{-1} which represent symmetric vibration of $–CH_2$- and asymmetric vibration of $–CH_3$, respectively. The same characteristic peaks are also seen in octadecyl trimethoxysilane spectra. In addition, the presence of ~1467 cm^{-1}, ~1268 cm^{-1}, ~1977 cm^{-1} in both the sol-gel C18 FPSE media and octadecyl trimethoxysilane strongly suggests the successful integration of octadecyl moieties into the sol-gel network. The substantial reduction of the O-H stretching vibrations (at 3325 cm^{-1}) in sol-gel C18 coated FPSE media compared to uncoated cotton (100% cellulose) indicates the chemical integration of the sol-gel C18 network to the cellulose structure via condensation. Due to the chemical integration of sol-gel sorbent to the substrate surface, the resulting FPSE media offer remarkably superior thermal, solvent and chemical stability than its commercial counterparts such as SPME, SBSE etc.

3.2 Optimization of the FPSE procedure

In order to achieve accurate and sensitive chromatographic quantification of the trace PAHs in the water samples, the optimum conditions for using sol-gel C18 coated FPSE media were

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investigated. Several conditions affecting the extraction efficiency were optimized, including extraction time, sample volume, eluting solvent, elution time, volume of organic modifier, and salt concentration. Optimization experiments were performed using a standard aqueous solution of PAHs containing 0.10 μg/ml of four PAHs each, to ensure the comparable level of responses to each compound.

3.2.1 Optimization of sample volume

To obtain high enrichment factors and high recoveries for all PAHs, the initial sample volume should be as large as possible. Therefore, different volumes (5 mL, 10 mL, 15 mL, 20 mL) of an aqueous solution were investigated. It was found that the highest extraction efficiency was obtained with a sample volume of 10 mL, as shown in Figure 5 (a). As the sample volume was increased up to 15 mL, recovery increased and after 15 up to 20 mL no obvious change was observed, after 20mL recovery decreased up to 50 mL, inferring that the extraction efficiency was insufficient at volumes above 20 mL. Therefore, the initial sample volume was set at 15 mL for future FPSE protocol.

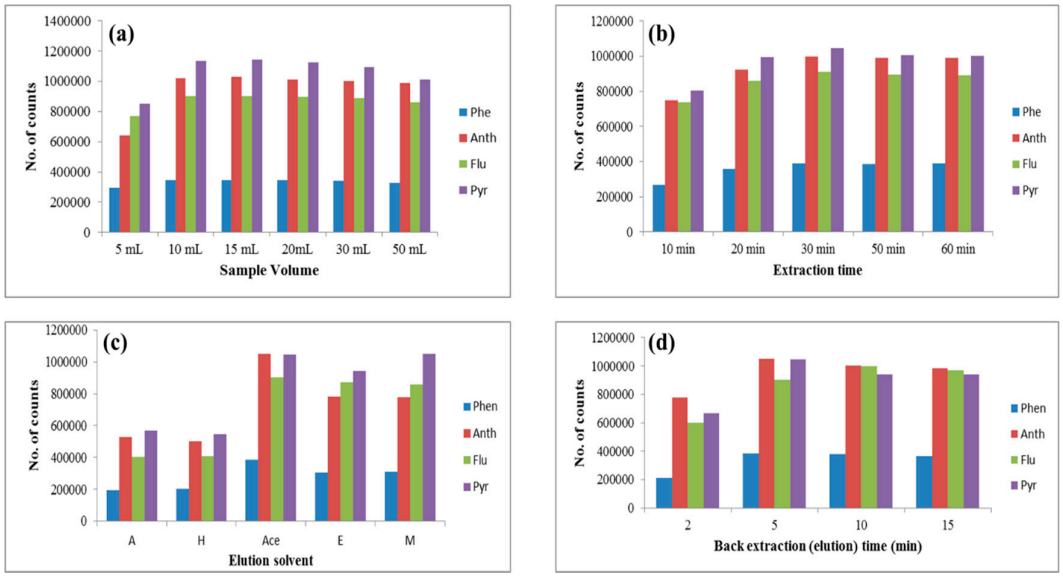

Figure 5. (a) Effect of sample volume; (b) Effect of extraction time; (c) Selection of the best elution solvent; (d) Optimization of back-extraction time. Extraction conditions: extraction time: 30 min; eluent solvent: acetonitrile; volume of elution solvent: 300 μL desorption time: 5 min

3.2.2. Optimization of extraction time.

Another key factor that effects extraction efficiency is the extraction time and it is imperative that this factor be thoroughly investigated. The extraction time was set from 10 to 50 min. As shown in Figure 5 (b), recoveries of all four PAHs increased with the extraction time as it was altered from 10 to 30 min, and then remained unchanged even when the time was increased up to 50 min, inferring that the extraction equilibrium was achieved at about 30 min. Thus 30 minutes were selected as the extraction time of FPSE protocol.

3.2.3. Optimization of desorption solvent and time

As far as the FPSE protocol is concerned, PAHs desorption from the sol-gel C18 coated FPSE media can significantly affect the sensitivity of the PAHs extraction. Thus, appropriate elution solvent plays a key role in the process. Because of the properties of PAHs, acetone (A), n-hexane (H), acetonitrile (Ace), ethanol (E) and methanol (M) were selected as potential eluting solvents in this

experiment. As shown in Figure 5 (c), acetone and n-hexane are poor eluents for PAHs. Methanol yields the highest recovery for Pyr, whereas acetonitrile is preferable for recovery of all four PAHs. Therefore, acetonitrile was chosen as the elution solvent and was used for further studies. Meanwhile, the elution efficiency also relies on the volume of the elution solvent. As shown in Supplementary Figure 1 for most of the PAH, the recovery increased as the eluent volume increased from 100 μL to 300 μL, which remains nearly unchanged if the volume is further increased from 300μL to 700 μL after that dilution effect leads to recovery decrease. To get high recoveries by as far as possible less solvent, 300 μL of acetonitrile was selected for desorption.

In order to resolve any possible carryover problem and avoid the loss of PAHs, the sonication desorption time was further optimized. The process of desorption was carried out in an ultrasonic bath with desorption times of 1, 3, 5 and 10 min. The results shown in Figure 5 (d) prove that the peak areas of PAHs increased as the desorption time increased from 1 min to 5 min, but remained unchanged as the desorption time was increased further. Thus, 5 min was sufficient to achieve maximum desorption.

3.2.4 Effect of salt concentration and organic modifier

Salt ions in the sample might also affect FPSE by the competitive interaction between the salting-in and the salting-out effect. The salting-out effect causes the analyte to enhances its partition onto the sol-gel C18 coated FPSE media by decreasing its solubility in water, while the salting-in effect has the opposite effect. Thus, the effect of the addition of salt to the samples was investigated and shown in Supplementary Figure 2. No obvious change was observed for the recoveries with KCl at 0–100 mM, indicating that salt ion addition does not affect extraction efficiency. This is probably due to low polarities of all four PAHs. Thus, no salt was added in the following experiment.

Addition of organic modifier, such as methanol might promote the extraction efficiency of FPSE for PAHs, by preventing the FPSE C_{18} carbon chains from cross-linking and thus, contacting with the target analytes completely. It was found that methanol addition (0–3 mL) did not cause any changes in extraction efficiency as shown in Supplementary Figure 3. This was probably because of the fact that FPSE media coated with C18 hydrophobic long chains spreads out well in the form of a uniform film on both sides of cellulose fabric substrate, due to strong chemical bonding between the sol-gel sorbent and the fabric substrate. Therefore, no organic modifier is needed. This indicates that the sol-gel C18 coated FPSE media is stable in various solutions, and the extraction efficiency is independent of the salinity and organic modifier.

3.3 Regeneration and reusability of sol-gel C18 coated FPSE media

The durability of sol-gel C18 coated FPSE media was also investigated by extracting PAHs from a water sample for 30 times. The FPSE media was regenerated with sonication in 10 mL acetonitrile for 15 min. The extraction efficiency of the sol-gel C18 coated FPSE media was almost unchanged after 30 extraction procedures, and the results are shown in Supplementary Figure 4. The results indicate that the sol-gel C18 coated FPSE media can be repeatedly used for extraction.

3.4 Analytical performance

All data were subjected to strict quality control procedures. The linearities of the chromatographic responses of the protocol were tested with calibration standards at seven concentration levels ranging from 0.01 to 10 ng/ml. Good linearities were observed for the four target PAHs, with all the correlation coefficients (R^2) above 0.99. Limits of quantification (LOQs) (signal-to-noise ratio = 10) and limits of detection (LODs) (signal-to-noise ratio = 3) of the four target compounds are shown in Table 1.

Table 1. Linear range, linearity curve, correlation coefficients, LODs and LOQs for the determinatio n of PAHs (n =5)

Analyte	Linear range (ng/ml)	Linearity curve	R^2	LOD (pg/ml)	LOQ (pg/ml)
Phen	0.010-10	y = 34591x + 772	0.9997	1	3
Anth	0.010-10	y = 104873x + 2147	0.9997	0.1	0.3
Flu	0.010-10	y = 90258x + 247	0.9987	0.7	2.1
Pyr	0.010-10	y = 114921x + 497	0.9983	0.4	1.2

The recovery studies were performed on 15 mL of deionized organic-free water spiked with known levels (final concentration 5 ng/ml of each) of the four PAHs respectively (five replicates). Mean recoveries ranged from 88.1 % to 90.5 % for all the target compounds. The mixture of standards was analyzed for five times within a day. The relative standard deviations (RSD) of concentrations for all the targets ranged from 1.1% to 4.1% (Table 2), demonstrating the high precision of the analytical protocol. In order to ensure the accuracy of the analysis, all samples were replicated five times, and the final concentration averages were used.

Table 2. Precision, accuracy and recovery of four selected PAHs (5 ng/ml of each PAH) in spiked deionized organic-free water (n = 5)

Analyte	Phen	Anth	Flu	Pyr
Precision (RSD%)				
Intra-day	3.6	2.2	1.8	1.1
Inter-day	4.1	2.5	1.9	2.0
Accuracy (%)				
Intra-day	90	91	92.1	92.8
Inter-day	89.4	89.3	92	91
Recovery (%)(RSD%)	88.4 (3.8)	88.1 (2.4)	90.5 (1.8)	90.1 (1.4)

3.5 Mathematical model for predicting extraction efficiency (absolute recovery, %)

A mathematical model was created for sol–gel C18 coated FPSE media that can be used as a predictive tool to assess the extraction efficiency (expressed as the absolute recovery) of analytes on sol–gel C18 coated FPSE media using their log Kow values. A carefully chosen test mixture was created to develop the mathematical model, consisting of 10 compounds representing different polarity and functionality (log K_{ow} values ranging from 0.3 to 5.07). The selected test compounds included piperonal (PIP), phenol (PHE), furfuryl alcohol (FA), benzodioxole (BDO), naphthalene (NAP), 4-nitrotoluene (4NT), 9-anthracene methanol (9AM), 1,2,4,5-tetramethyl benzene (TMB), triclosan (TCL), and diethylstilbestrol (DESB). Extraction recovery value of each compound from an aqueous solution of the test mixture was determined and the values were plotted against their

log K_{ow} values to obtain a second order mathematical model: **Extraction efficiency (absolute recovery, %) = -2.274875 + 20.816015*log K_{ow} -4.1478973*(log K_{ow}-2.737)²**. This second order mathematical model can be used to predict the extraction efficiency of sol–gel C18 coated FPSE media for a given analyte using its log K_{ow} value. A graphical representation of the model is shown in Figure 6.

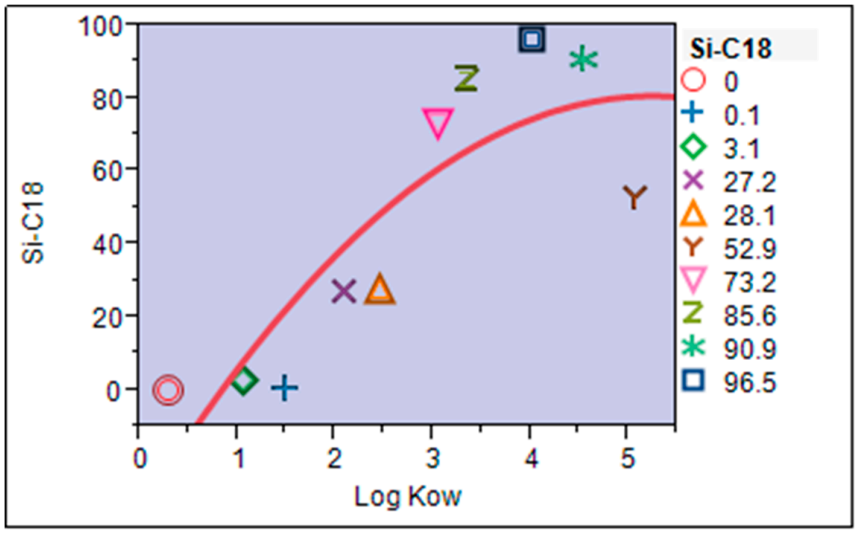

Figure 6. Correlation curve between absolute recovery and logarithmic values of octanol-coefficients of analytes with wide polarity range.

The predicted recovery values for four PAHs using the mathematical model and their actual recovery values are given in Table 3.

Table 3. Data demonstrating model predicted absolute recovery (%) and actual recovery (%) obtained by FPSE-HPLC-FLD method

Compound	Log Kow	Expected recovery (%)	Actual recovery (%)
Phen	4.46	78.30	88.4
Anth	4.45	78.24	88.1
Flu	5.16	80.85	90.5
Pyr	4.88	80.32	90.1

For all of the target PAHs, the actual extraction recovery values were found higher than the predicted values obtained from the model. This was attributed to the strong PAH– sol-gel C18 hydrophobic interactions in addition to efficient trapping of PAHs on sol-gel C18 extraction media having high primary contact surface area, high loading of sol-gel nanocomposite sorbent in the form of ultrathin film as well as the permeability of the extraction device that mimics a solid phase extraction disc (characterized with exhaustive extraction).

3.6 Application to real water samples

Subsequently, the FPSE-HPLC-FLD protocol developed was applied for PAHs determination in real environmental water samples: bore well water, river water, rain water and factory wastewater. Quintuplicate analyses were performed and the concentrations found for the target PAHs are summarized in Table 3. In the four types of environmental water samples, the target four PAHs were not detected in bore well water and rain water. Phen and Flu were detected in two river water

samples and all four target PAHs were found in each of the two metal-fabrication factory wastewater samples (Table 4).

Table 4. The four selected PAHs concentrations detected in real water samples (n = 5)

Sample	Phen (ng/ml)	Anth (ng/ml)	Flu (ng/ml)	Pyr (ng/ml)
A[a]	n.d.	n.d	n.d	n.d
B[a]	n.d	n.d	n.d	n.d
C[b]	7.8	n.d.	5.6	n.d
D[b]	8.8	n.d.	6.8	n.d
E[c]	n.d	n.d	n.d	n.d
F[c]	n.d	n.d	n.d	n.d
G[d]	11	7.8	7.5	5.8
H[d]	4.1	3.7	7.7	3.0

a: borewell water, b: river water, c: rain water, d: factory waste water, n.d.: not detected

The two river water samples, collected from two different locations in the Chakki River in Pathankot were analyzed. At one outlet point of metal factory wastewater, Phen, Anth, Flu, Pyr were detected at 11 ng/mL, 7.8 ng/mL, 7.5 ng/mL and 5.8 ng/mL respectively, while at second outlet point, Phen, Anth, Flu, Pyr were detected at 4.1 ng/mL, 3.7 ng/mL, 7.7 ng/mL and 3.0 ng/mL respectively, (shown in Table 3); suggesting that the PAHs came mainly from the wastewater. These results indicated that the method could be successfully applied to PAH analysis in real water samples. The typical chromatograms of a river water samples and a metal factory waste water samples are presented in Figure 7**.**

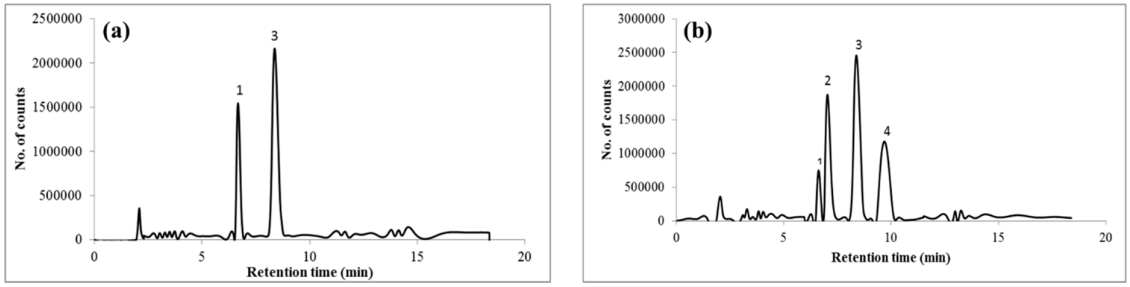

Figure 7. Chromatogram representing analysis of real environmental samples: (a) Chakki river water sample-2; (b) factory wastewater sample-2.

3.7 Comparison of sol-gel C18 coated FPSE media with other sorbent materials

The performance of the new analytical protocol with the sol-gel C18 coated FPSE media as adsorbent was also compared with other materials reported in the literature (as shown in Table 5).

Table 5. Comparisons of the analytical performance of the method developed with reported methods in the literature.

Method	Sorbent material	Sorbent preparation time (hours)	LOD (pg/ml)	References
MSPE	TPA-functionalized MNPs	37.5	0.04–3.75	**[53]**
μ-SPE	Functionalized graphene sheet	29.5	0.8-3.9	**[54]**

SPE	Cotton fiber	0.5	0.1-2	
MIPs-SPE	Imprinted sol-gel adsorbent	38	5.2-12.6	[55]
FPSE	Sol-gel C_{18} (Cellulose)	24	0.1-1	Current work

Abbreviation: MIPs: molecularly imprinted polymers; MSPE: magnetic solid-phase extraction; TPA-functionalized MNPs: triphenylamine functionalized magnetic microspheres.

The time required in sorbent preparation and the LOD values were compared. The LOD values achieved in the present research are lower than those reported in the literature. Furthermore, sol-gel C18 coated FPSE media as a sorbent has obvious advantages: preparation of sorbents often require several days to complete the synthesis process, involve dozens or hundreds of mL of organic solvent consumption, time consuming and may cause environment pollution, whereas sol-gel C18 coated FPSE media, an advanced inorganic-organic hybrid material with tunable porosity, selectivity, thermal and chemical stability, high reproducibility and solvent resistant, requires less volume of organic solvent, fast, and a green analytical endeavor. Therefore, the proposed FPSE-HPLC-FLD protocol is proven to be a green, convenient, efficient and reliable method for the pre-concentration of trace PAHs from water samples.

5. Conclusions

In summary, for the first time, sol-gel C18 coated FPSE media was directly and successfully applied as a selective adsorbent for PAH analysis in real environmental water samples. The LODs obtained for four target PAH compounds using the novel analytical protocol were 0.1–1 pg/mL. Compared to traditional SPE methods, the sol-gel C18 coated FPSE media are easy to prepare and simple to regenerate for recurring usage which meets the need for rapid analysis. In addition, the costs of the preparation of the sol-gel C18 coated FPSE media are economical, and organic solvent consumption is minimal. Sol-gel C18 FPSE media can also be regenerated and reused more than 30 times. The FPSE media are also degradable, and thus, more environment friendly. Utilizing the unique attributes of FPSE, a novel, simple, efficient, fast, sensitive, green, economical and reliable FPSE-HPLC-FLD protocol is presented for trace level determination of four environmentally important PAHs, and this protocol allow wide applications in health-related water contamination studies and offers new analytical capabilities for water quality assurance, to routinely monitor the presence of PAHs in water samples in order to ensure water quality, safety and consumer protection.

Supplementary Materials: The following are available online at www.mdpi.com/link, Figure S1: Effect of eluent solvent volume: Extraction conditions: sample volume, 15 mL; extraction time: 30 min; eluent solvent: acetonitrile; sonication desorption time 5 min. Figure S2: Effect of salt concentration: Extraction conditions: sample volume, 15 mL; extraction time: 30 min; eluent solvent, acetonitrile; volume of elution solvent, 300 µL desorption time, 5 min. Figure S3: Effect of organic modifier volume: Extraction conditions: sample volume, 15 mL; extraction time: 30 min; eluent solvent, acetonitrile; volume of elution solvent, 300 µL desorption time, 5 min. Figure S4: Regeneration and reusability of sol-gel C18 coated FPSE media. Extraction conditions: sample volume, 15 mL; extraction time: 30 min; eluent solvent: acetonitrile; volume of elution solvent: 300 µL desorption time, 5 min.

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Conflicts of Interest: The authors declare no conflict of interest.

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