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Application of multi-walled carbon nanotube-agarose/chitosan composite film microextraction-HPLC-UV to the determination of anthracene in lake water

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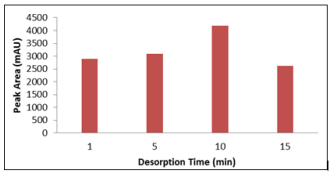
GRAPHICAL ABSTRACT



Effect of desorption time on MWCNT-ACFME of PAHs from spiked deionized water sample.

ABSTRACT

This study investigates a new approach in μ-solid phase extraction (μ-SPE) combined with high performance liquid chromatography ultraviolet detection (HPLC-UV) for the analysis of polycyclic aromatic hydrocarbon (PAHs) namely anthracene in water samples. The μ-SPE method utilized multi-walled carbon nanotubes (MWCNTs) immobilized in agarose-chitosan film to serve as the sorbent. The presence of amino functional group in chitosan offered a good dispersion of MWCNTs in a agarose/chitosan matrix and prevent deactivation of sorbent via agglomeration. Ionic interaction between amino groups of chitosan with MWCNTs produce more stable composite film and prevent leaching of sorbent during application. The film was prepared by mixing MWCNTs, agarose and chitosan, followed by drying in an oven. The prepared film was characterized by Fourier transform infrared spectroscopy (FTIR). Microextraction of anthracene was performed by inserting a hypodermic needle through circular MWCNTs-agarose/chitosan film discs (5 mm diameter) and the assembly was dipped into an agitated sample solution prior to HPLC-UV analysis. Important parameters studied included extraction time, desorption time and amount of adsorbent loading. Calibration curves prepared showed good linearity with $r \geq 0.9932$ over the concentration range of 10−500 μg L^{-1}. The limits of detection (LODs) and limits of quantification (LOQs) of anthracene were 0.657 μg L^{-1} and 2.192 μg L^{-1}, respectively, with satisfactory recoveries in the range of 85.23% to 119.05% and acceptable reproducibility, RSD ≤ 10.90%. The proposed method showed good sensitivity and was successfully employed in the analysis of PAHs in lake water samples.

Keywords: agarose-chitosan film, MWCNTs, micro-solid phase extraction, PAHs, HPLC-UV

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) defined as a group of aromatic hydrocarbons with two or more fused benzene rings. PAHs also have proved to be the main components responsible for effect on organisms, because of their carcinogenic, mutagenic and toxic effects [1]. PAHs are one of the most common organic pollutants that present in water. They also show a high persistency and low biodegradability characteristic due to their highly hydrophobic nature which may later accumulate in a concentration that toxic and unhealthy to the environment [2]. Anthracene is a solid PAHs consisting of three benzene rings (Figure 1) and derived from coal tar. Anthracene can be found in the form of white to yellow crystalline solid. Anthracene has been used as diluents for wood protection products, an insecticide or a fungicide and has been identified in surface and drinking water [3]. Contamination of these hazardous compounds into surface water and ground water can present serious health effect on humans. Hence, the development of new methods for the monitoring of trace levels of PAHs from varied environmental matrices such as water is important.

Water is one of the most essential sources in our life and the control of contaminated compounds such PAHs in water is a real necessity nowadays.

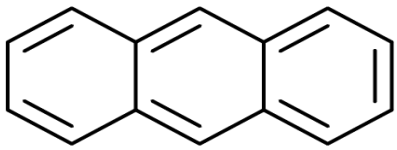


Fig. 1 Molecular structure of Anthracene

2. EXPERIMENTAL

The film was characterized using Fourier-transform infrared (FT-IR). All analyses were performed using a high performance liquid chromatography (HPLC) (Agilent Technologies, Milan, Italy) coupled with a ultraviolet detection (Agilent Technologies). The chromatographic separation was carried out using PHENOMENEX C_{18} column (4.6 x 150 mm, 5 μm) and acetonitrile-water mixture (80:20) as mobile phase with flow rate of 0.2 mL min^{-1}. The detection wavelength was set up at wavelength 254 nm.

2.1. Chemical and Material

Anthracene was obtained from Sigma-Aldrich (St. Louis, USA). Stock solution of 1000 ppm of anthracene was prepared in acetonitrile. HPLC grade acetonitrile was obtained from Merck (Darmstadt, Germany). Acetic acid glacial was from QRec (New Zealand). Agarose (analytical grade) and chitosan were obtained from Promega Corporation (Madison, USA) and Sigma-Aldrich (St. Louis, USA) respectively. Multi-walled carbon nanotubes (MWCNTs) with specific surface area > 233 m^2g^{-1}, purity > 95%, 8-15 nm outer diameter × 50 μm in length were purchased from Sun Nanotech (Jiangxi, China). Lake water sample was obtained from UTM Lake, Johor Bahru.

2.2. Preparation of Multi-walled Carbon nanotube Incorporated Agarose/chitosan Composite Film

The preparation method followed was based on the work by Wan Ibrahim, *et. al* [4]. Agarose (0.10 g) was added in 10 mL of deionized water and chitosan (0.10 g) was added in 10 mL of 0.1 M acetic acid respectively. Both mixtures were heated (90 ºC) and stirred until completely dissolved. The agarose solution was added drop by drop into the chitosan solution and was continuous heated at 90 ºC and stirred for further 30 min. Then, MWCNT (40 mg) was added to the solution and was continuous stirred for another 30 min. The mixture (10 mL) was poured into a Petri dish (50 mm in diameter) and the solution was allowed to cool at room temperature for 30 min. The gel was dried in an oven at 60 ºC for 48 hours. Next, the film formed was punched into circular discs of 5 mm diameter to be used as adsorbent during the microextraction process.

2.3. Preparation of Anthracene Stock Solution

Stock solution of 1000 ppm was prepared for anthracene by weighing 0.005 g of anthracene standard into a 5 mL volumetric flask and dissolve in HPLC grade acetonitrile and made up to the mark. Standard working solutions (100 ppm and 10 ppm) of anthracene standard were prepared by dilution from the stock solution. The stock solutions and diluted solutions were stored in refrigerator at 4ºC for further usage

2.4. Multi-walled Carbon nanotube Incorporated Agarose/chitosan Composite Film Microextraction (MWCNT-ACFME)

Multi-walled carbon nanotube incorporated agarose/chitosan composite film (Agr-Ch-MWCNT) was conditioned in isopropanol (1 min) and deionized water (1 min) before dipping into the sample solution. A hypodermic needle was used to pierce the Parafilm and 5 pieces of film, which alternately separated by silicon septum. Next, the assembly was dipped into a sample solution (10 mL) and immediately sealed with the Parafilm. The solution was stirred at 750 rpm for 20 min to transport the targeted analytes to the adsorption sites on the films. After the extraction, the film were removed and placed in 200 μL isopropanol and ultrasonicated (10 min) for desorption process. 2μL of the final extract will be injected into the HPLC-UV for analysis.

3. RESULTS AND DISCUSSION

3.1 Characterization of film by FT-IR

MWCNTs-agarose/chitosan composite film and agarose-chitosan film were characterized using FT-IR. Based on the FT-IR analysis (Figure 2), (Table 1) there are the presences of O-H bonds for both MWCNTs-agarose/chitosan film and agarose-chitosan film with wavelength of 3281.85 cm^{-1} and 3356.04 cm^{-1} respectively. Addition of MWCNTs into agarose-chitosan film show a shift on O-H bonds from 3356.04 cm^{-1} to 3281.85 cm^{-1} indicating that MWCNTs had interacted with agarose-chitosan film through π-bond interaction with amino and hydroxyl groups of agarose and chitosan [4].

Table 1: FTIR data of spectrum a, b, c, and d.

Spectrum	Type of film and starting material	OH stretching, cm^{-1}	NH stretching, cm^{-1}	NH bending, cm^{-1}	C=O stretch, cm^{-1}
a	MWCNTs-agarose/chitosan	3281.85	-	1635.36	-
b	Agarose-chitosan	3356.04	3276.68	1636.42	-
c	Chitosan	3446.82	-	1638.33	-
d	Agarose	3435.98	-	-	-
e	MWCNTs	3451.21	-	-	1631.50

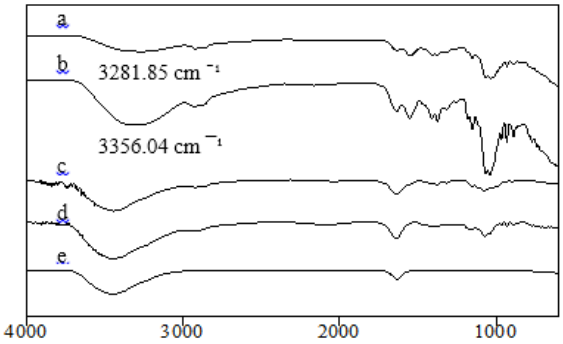


Fig. 2 FT-IR spectra of different types of films. (a=MWCNTs-agarose/chitosan film, b=agarose-chitosan film). FT-IR spectra of starting materials. (c=Chitosan, d=Agarose, e=MWCNTs)

3.2 HPLC Identification

The standard solution of PAHs was injected into the HPLC injection port at a concentration of 10 ppm for anthracene to identify based on the retention time of the peak. The result (Figure 3) showed that retention time for anthracene was at 6.05 min.

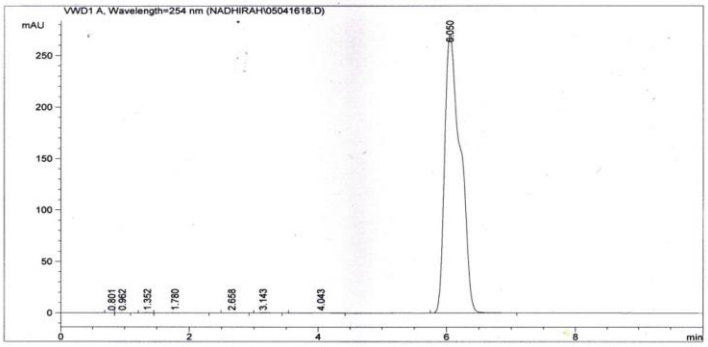


Fig. 3 HPLC chromatogram for anthracene. HPLC Conditions: Mobile phase composition ACN: H_2O 80:20, v/v and wavelength of 254 nm and flow rate of 0.2 mL min^{-1}.

3.3 Percentages of MWCNTs

Different amount of MWCNTs incorporated in agarose-chitosan film in the range 0.0-0.4 % (w/v) were studied. In this analysis, MWCNTs acted as an adsorbent for anthracene extraction. It was expected that MWCNTs adsorbed anthracene by the coactions of delocalized π-bond interaction and physical adsorption [5]. As shown in Figure 4, the peak area of anthracene increased with the concentration of MWCNTs from 0.0 % to 0.2 % and there is no significance difference between 0.2 % and 0.4 % MWCNTs.

From the investigation, it was shown that agarose-chitosan film (AgrCh) also contributes towards the extraction of PAHs. Based on FT-IR analysis, this might due to the presence of O-H bonds (3356.04 cm^{-1}) and N-H bonds (stretching N-H bond: 3276.68 cm^{-1}; bending N-H bond: 1636.42 cm^{-1}) in AgrCh film to interact with PAHs compounds through π-π interaction (Figure 2), (Table 1). Therefore, 0.4 % of MWCNTs was selected as the optimum condition and used subsequently for further extraction process.

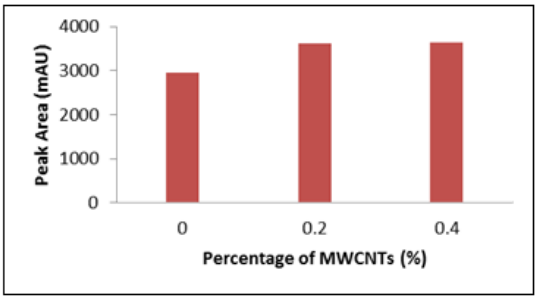


Fig. 4 Effect of percentage of MWCNTs on MWCNT-ACFME of anthracene from spiked deionized water sample. Extraction condition: 10 mL water sample, 750 rpm stirring rate, 10 min extraction time and 5 min desorption time. HPLC condition: flow rate 0.2 mL min^{-1} with mobile phase composition of ACN:H_2O 80:20, v/v and wavelength of 254 nm.

3.4 Extraction Time

The MWCNT-ACFME is not an exhaustive extraction but it depends on equilibrium time. Therefore, extraction times in the range of 10-40 min were studied to determine the equilibrium time required for anthracene to be adsorbed on adsorbent film. Figure 5 shows the extraction time profile of anthracene. From the study, it was considered that 20 min is needed to reach extraction equilibrium between composite film and aqueous phase. Therefore, extraction time of 20 min was selected for subsequent experiments. There is a significant decrease in extraction time after 20 min. This might due to Agr-Ch-MWCNT film was only capable for extraction of analyte within its framework and it was less affected by longer extraction time.

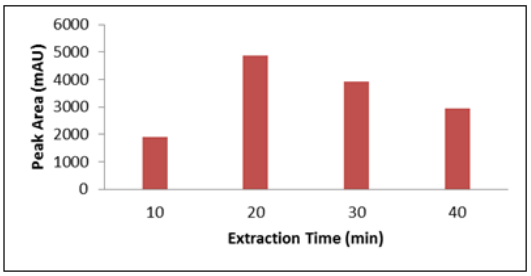


Fig. 5 Effect of extraction time on MWCNT-ACFME of anthracene from spiked deionized water sample. Extraction condition: 10 mL water sample, 750 rpm stirring rate, 0.4 % MWCNTs, and 5 min desorption time. HPLC condition: flow rate 0.2 mL min^{-1} with mobile phase composition of ACN:H_2O 80:20, v/v and wavelength of 254 nm.

3.5 Desorption Time

Ultrasonification method was used to desorp anthracene from the hydrophobic MWCNTs as the technique was suitable to be applied for reversible adsorption [6]. The effect of desorption time in the range of 1 - 15 min of ultrasonification was investigated. It was found that maximum desorption efficiency of anthracene was achieved within 10 min of ultrasonification (Figure 6). There is a decreased of peak area was observed beyond 10 min of ultrasonification might due to temperature increased during long time of sonication that probably will degrade the analytes. Therefore, desorption time of 10 min with ultrasonification was chosen and used for further experiment.

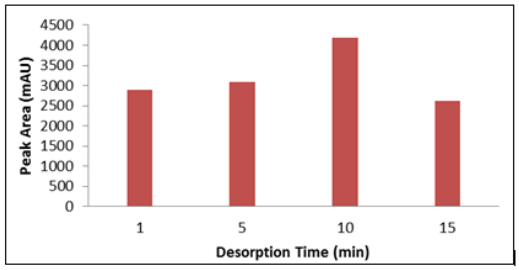


Fig. 6 Effect of desorption time on MWCNT-ACFME of PAHs from spiked deionized water sample. Extraction condition: 10 mL water sample, 750 rpm stirring rate, 0.4 % MWCNTs, and 20 min extraction time. HPLC condition: flow rate 0.2 mL min^{-1} with mobile phase composition of ACN:H_2O 80:20, v/v and wavelength of 254 nm.

3.6 Validation of MWCNT-ACFME

Matrix matched calibration was carried out using lake water sample spiked with targeted analytes in the range of 10 to 500 μg L^{-1} for anthracene. Good linearity (Figure 7) was obtained in the specified concentration ranges with correlation coefficients, $r \geq 0.9932$. The LODs and LOQs obtained were 0.657 and 2.192 μg L^{-1} respectively. The LODs obtained indicated that MWCNTs is a good sorbent for anthracene, where the sorption is supported by combination of hydrophobic and π-π interaction.

Relative recovery was studied for the MWCNT-ACFME system. The sample was spiked at 100 and 400 μg L^{-1} for anthracene. Anthracene was extracted using the developed MWCNT-ACFME method. The result (Table 2) showed average relative recoveries in the range of 85.23 % to 119.05 % and acceptable reproducibility with relative standard deviations (RSDs) of ≤ 10.90 %. This revealed that matrix effect was negligible and thus the MWCNT-ACFME method could be employed in the analysis of anthracene from lake water sample.

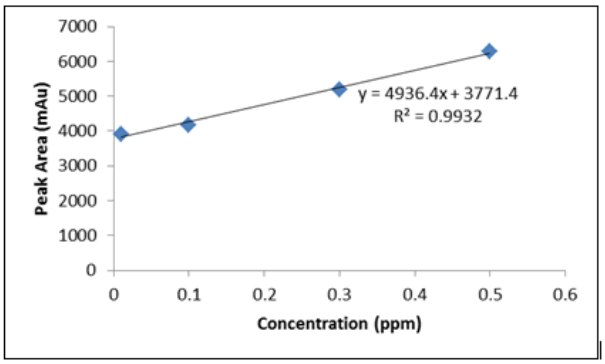


Fig. 7 Matrix-matched extraction calibration plot for anthracene. Extraction conditions: 10 mL water sample, 750 rpm stirring rate, 20 min extraction time and 10 min desorption time. HPLC condition: flow rate 0.2 mL min^{-1} with mobile phase composition of ACN:H_2O 80:20, v/v and wavelength of 254 nm.

Table 2 Relative recovery studies of MWCNT-ACFME using spiked lake water sample.

PAH	Calibration concentration, μg L^{-1}	Average relative recoveries, % (RSD, %)
Anthracene	100	85.23 (10.90)
	400	119.05 (10.25)

3.7 Application of MWCNT-ACFME to the analysis of Real Sample

The optimized and validated MWCNT-ACFME method was applied to the analysis of anthracene in lake water samples. The result (Figure 8) showed that anthracene was detected at lake water sample at very low concentration level which not exceeds the allowed limit.

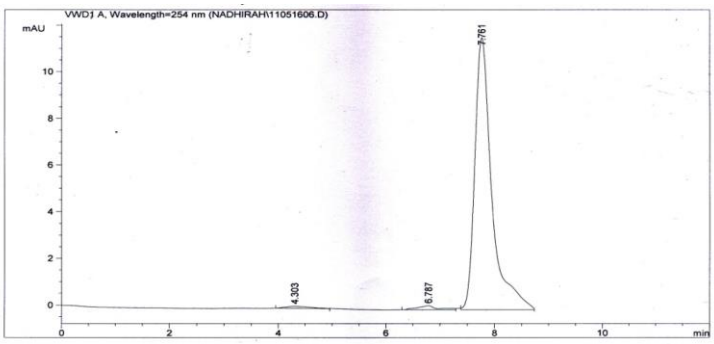


Fig. 8 HPLC chromatogram of UTM lake water sample. HPLC conditions: flow rate 0.2 mL min^{-1} with mobile phase composition of ACN:H_2O 80:20, v/v and wavelength of 254 nm.

4. CONCLUSION

This study proved that MWCNT-ACFME is suitable for trace analysis of PAHs such as anthracene. This MWCNT-ACFME method has eliminated centrifugation and filtration process in classical separation techniques. MWCNTs were used as a dispersive sorbent. The calibration curves prepared showed acceptable linearity with $r \geq 0.9932$ over the concentration range of 10 – 500 μg L^{-1}. The LODs (S/N = 3) and LOQs (S/N = 10) of anthracene were 0.657 μg L^{-1} and 2.192 μg L^{-1}, respectively, with good recoveries obtained in the range of 85.23% to 119.05 %. The method also showed acceptable reproducibility (RSD ≤ 10.90 %, *n* = 3). The satisfactory sensitivity demonstrated in this study showed that MWCNT-ACFME is capable to act as new environmental friendly μ-SPE method that is able to separate and identify anthracene effectively. This method offers advantages in the field of analytical chemistry as it is cheap and avoids the high cost involve for chemical waste disposal and also contribute less to environmental pollution as the use of organic solvent is minimized. This method also might be able to help to minimize the PAHs exposure in aqueous media and directly can protect human and aquatic life from exposed to the carcinogenic and mutagenic of PAHs.

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