J Fluoresc (2014) 24:787–793
DOI 10.1007/s10895-014-1352-9

ORIGINAL PAPER

Novel Method for Determination of Anthracene by Coupling Dispersive Liquid-Liquid Extraction to First-Derivative Synchronous Spectrofluorimetry

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Received: 10 November 2013 / Accepted: 27 January 2014 / Published online: 4 February 2014

Abstract A novel method could be adopted successfully for determination of anthracene in environmental samples, utilizing dispersive liquid-liquid extraction followed by first-derivative synchronous fluorimetry at a constant wavelength difference $\Delta\lambda$=165 nm, where a linear calibration curve was obtained in a concentration range of 0.5–100 ng mL^{-1} at 244 nm. The detection limit was 0.1 ng mL^{-1}. The method can be easily adopted for determination of anthracene in aqueous media including tap water and river water. The recoveries obtained were 85.40–108.02 %. The proposed method was validated according to International Conference of Harmonization (ICH) guide lines and successfully applied to determine anthracene in pure form and in water samples including real life water samples from different sources. All the results obtained were compared with those of published method, where no a significant difference was observed.

Keywords Anthracene · Dispersive liquid-liquid extraction · Derivative-synchronous spectrofluorimetry · Water samples

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants which are known for their teratogenic, carcinogenic and mutagenic potential [1, 2], consequently their monitoring is of great importance.

Anthracene (Fig. 1) is one of the smallest members of polycyclic aromatic hydrocarbons, consisting of three fused benzene rings [3]. It is a component of coal tar, generated from incomplete combustion of different organic materials as in case of motor vehicle traffic and forest fires [4, 5]. There are several routes of anthracene exposure including food, water, soil, dust and even occupational exposure. Thus, it is of great importance to develop rapid and sensitive methods for monitoring of such environmental pollutants.

Anthracene is one of the 16 unsubstituted PAH priority pollutants which have been identified by the U.S. Environmental Protection Agency (EPA). Therefore, several analytical methods have been reported for its determination, but most of them are gas chromatography (GC) and high-performance liquid chromatography (HPLC) methods [6, 7]. These methods are very accurate, but they suffer from several drawbacks, including that they are time consuming, expensive and need significant amounts of solvent and sample, furthermore, they need an experienced analytical chemist [5, 8].

Fluorescence spectroscopy is a technique of high sensitivity, as usually it determines analytes in ng mL^{-1} to pg mL^{-1} range, thus, it is well suited for environmental monitoring, especially, in case of polycyclic aromatic hydrocarbons which have high native fluorescence [9]. But, there are some drawbacks in conventional fluorescence spectroscopy, including the need for adjustment of excitation and emission wavelengths, which could be a problem for new substances when no excitation or emission wavelength is known. In addition to that, the emission spectra of many substances tend to be broad and not symmetric [9].

Synchronous fluorescence spectrometric technique provides an improvement of the fluorescence spectroscopy by simplification of fluorescence spectra and its well known band-narrowing effect [10].

Furthermore, there is no need to handle two different spectra (exCitation and emission), instead only one synchronous spectrum is obtained and by adjusting a suitable wavelength difference $\Delta\lambda$, where a good peak shape can be

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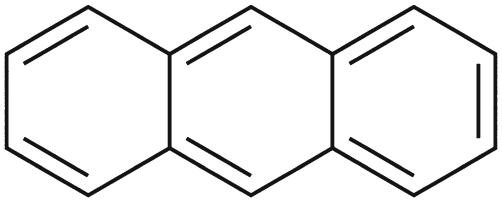

Fig. 1 Structure of anthracene

obtained in most of the cases [11]. Synchronous spectrofluorimetry has been used mainly for resolution of mixtures which were not easily resolved by conventional spectrofluorimetry [12–19].

The combination of synchronous and derivative fluorimetry enhances minor spectral features and allows more reliable identification of chemical species [20]. Also, this combination has been used to determine single compounds rather than mixtures in order to decrease matrix interference and to obtain better peak resolution [10].

Usually, analysis of water samples needs an extraction step in order to transfer the analyte from the aqueous phase to an organic phase, leading to more sample concentration. The problem of conventional liquid-liquid extraction technique lies in that, it is tedious, time-consuming and needs large volumes of organic solvents [21].

Therefore, several liquid-phase microextraction techniques emerged in the mid-to-late 1990s, including single drop microextraction (SDME), cloud point extraction (CPE) and homogenous liquid-liquid extraction (HLLE) [22]. Recently, in 2006, Assadi and co-workers introduced dispersive liquid-liquid microextraction [23], which has the advantage that it is rapid, easy to be performed and uses small amounts of organic solvent [22, 24].

Dispersive liquid-liquid microextraction has been always coupled to high-performance liquid chromatography (HPLC), gas chromatography (GC) and atomic absorption spectroscopy (AAS) [25–27] and never been coupled to spectrofluorimetry.

In the present paper, a new modification of dispersive liquid-liquid microextraction was successfully adopted in order to be able to analyze water samples containing anthracene, using simple synchronous spectrofluorimetry and consequently obtaining an easy, rapid and sensitive analysis procedure for anthracene determination.

Experimental

Chemicals and Reagents

Anthracene was obtained from Riedel-de Haën®, Sigma-Aldrich, Germany and certified as analytical standard and certified to contain 99.0 %. Methanol, ethanol, ethyl acetate, acetonitrile, hexane, cyclohexane, chloroform, benzene and dichloromethane were obtained from ADWIC, Egypt.

All chemical and reagents used through this work are of spectrofluorimetric analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word "water".

Instruments

Fluorescence was measured on Kontron SFM25 (BIO-TEK Kontron, Switzerland) spectrofluorimeter, equipped with a 150 W Xenon lamp and a photomultiplier detector.

The spectrofluorimeter was controlled by computer, using SFM25 software, the PMT voltage was adjusted at 400 V and the optimum scan speed of 300 nm s^{-1} was used.

All measurements took place in a standard 10 mm path length quartz cell, where the excitation and emission monochromators were scanned simultaneously with a constant difference $\Delta\lambda$=165 nm and a response time of 8 s.

The slit width of both monochromators was 5 nm and the synchronous spectra were recorded in an excitation scale.

Membrane filter (PTFE, 0.45 μm pore size) were used for filtration of environmental samples.

The hot plate and stirrer MTopo MS 300HS was used for heating and stirring.

Standard Solutions

Standard Solutions of Anthracene

Stock standard solution of anthracene, having a concentration of 0.5 mg mL^{-1} was prepared in methanol and then further diluted with the same solvent giving a solution having a concentration of 100 ng mL^{-1} to be used as a working standard solution.

Procedure

General Procedure

Aliquots of anthracene standard working solution were added to a series of 10.0 mL volumetric flasks and then diluted with methanol to obtain a concentration range of 0.5–100 ng mL^{-1}.

The excitation and emission spectra were recorded at 235 nm and 392 nm wavelengths, respectively and the synchronous spectra were recorded at a constant wavelength difference $\Delta\lambda$=165 nm.

In all cases, the blank spectra were drawn and subtracted by the software from the corresponding spectra, first-derivative (D^1) spectra were obtained using 25 experimental smoothing points and the measurements were done at 244 nm as the vertical distance from the peak to the baseline.

Anthracene sample concentrations were determined by constructing calibration curves and adopting regression equations.

Dispersive Liquid-Liquid Extraction Procedure

By a simple modification in the dispersive liquid-liquid micro-extraction technique, anthracene could be extracted from aqueous samples.

To 10.0 mL of aqueous anthracene sample, 3.0 mL dichloromethane were added directly into a 50 mL beaker, stirred at 1,500 rpm using a magnetic stirrer for 5.0 min, where dichloromethane was completely dispersed in the aqueous sample throughout this time. The mixture was then left for 1 min to separate into two layers and the lower organic layer (dichloromethane) was carefully collected by withdrawing it by using a conventional 5.0 ml syringe.

The collected dichloromethane layer was evaporated utilizing a hot plate and the residue was reconstituted in 10.0 ml methanol to be measured spectrofluorimetrically.

Sample Collection

Tap water was used directly from the tap and river water, collected from Nile River in Cairo at a place near Tahrir Square in amber glass containers, was used after filtration using Membrane filters (PTFE, 0.45 μm pore size).

Analysis of Spiked Tap and River Water

Tap water was spiked with different amounts of anthracene and the mentioned procedure ("General Procedure" Section and "Dispersive Liquid-Liquid Extraction Procedure" Section) was adopted.

Any impurities in river water that may interfere with the measurement process were firstly removed by filtration, using 0.45 μm membrane filters, then spiked with different amounts of anthracene and the mentioned procedure ("General Procedure" Section and "Dispersive Liquid-Liquid Extraction Procedure" Section) was utilized.

Analysis of Contaminated Environmental Water Samples

Water samples of three different sources which are likely to be contaminated with anthracene were collected and analyzed by the mentioned procedure.

The first one was collected from "shisha" (water pipe) and the mentioned procedure ("General Procedure" Section and "Dispersive Liquid-Liquid Extraction Procedure" Section) was adopted, utilizing 1.0 mL of the sample.

The second and third samples were collected from different industrial effluents and analyzed directly after extraction, using the mentioned procedure ("General Procedure" Section and "Dispersive Liquid-Liquid Extraction Procedure" Section).

Results and Discussion

Solvent Selection for Anthracene

Several solvents were tested in order to choose the most appropriate solvent which maximizes the fluorescence signal of anthracene.

Figure 2 shows a diagram representing different fluorescence intensities obtained by using various solvents, where anthracene in methanol exhibits the highest fluorescence intensity. The chosen solvent was utilized for determination of anthracene in order to reach low detection limits, which is essential for analysis of environmental samples.

Effect of Methanol Concentration

The effect of methanol concentration on the fluorescence intensity of anthracene was also investigated (Fig. 3), where it was found that 100 % methanol could yield the highest fluorescence intensity.

Optimization of Dispersive Liquid-Liquid Extraction Parameters

Effect of Extraction Solvent Type and Volume

In order to obtain highest extraction efficiency, different extraction solvents including dichloromethane, benzene, n-hexane, chloroform, and cyclohexane were tested, where there was no limitation considering the density of the used extraction solvent.

Both types of extraction solvents, lighter or heavier than water could be used without any problem as they rapidly separate from the aqueous solution after stirring and then they are easily collected by withdrawing it by a conventional syringe.

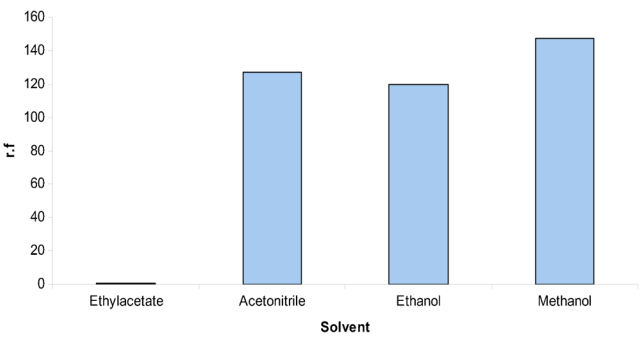

Fig. 2 Effect of different solvents on the fluorescence intensity of anthracene

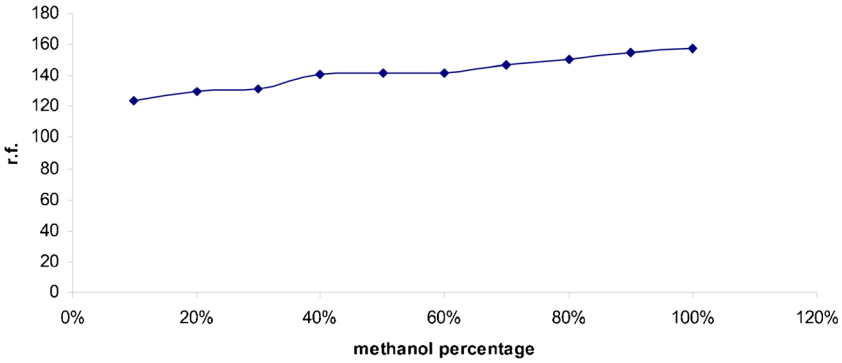

Fig. 3 Effect of methanol concentration on the fluorescence intensity of anthracene

This is a great advantage as a wide selection of extraction solvents could be obtained in contrast to other micro-extraction techniques in which the extraction solvent was limited by certain requirements [28].

Figure 4 shows different fluorescence intensities obtained by various solvents used for extraction of anthracene from 10.0 mL aqueous solution, having a concentration 50 ng mL^{-1}, where dichloromethane turned out to be the best extraction solvent.

To examine the volume of the extraction solvent, solutions containing different volumes of dichloromethane between 2 and 5 mL were subjected to the same dispersive liquid-liquid extraction procedure.

No improvement in fluorescence intensity was observed with increasing the extraction solvent-volume, consequently, 3 ml dichloromethane was chosen as an optimum extraction solvent-volume, since in case of smaller volumes the collection of the extraction-solvent was not easy, also the use of higher extraction-solvent volume is not environmentally friendly.

Effect of Extraction Time

In our work, extraction time is defined as time of stirring the extraction solvent together with the aqueous solution. The influence of the extraction time was evaluated in the range of 5–10 min with constant experimental conditions.

It was found that, increasing the time above 5 min has no significant effect on the extraction efficiency which is possibly due to the very large surface area produced between the extraction solvent and aqueous solution by stirring that permits a rapid transfer of anthracene from aqueous to organic phase [27]. This is one of the considerable advantages demonstrated by the dispersive liquid-liquid extraction technique.

In this method, the most time consuming steps are stirring of the immiscible mixture which takes 5 min and evaporating of dichloromethane which takes about 3 min.

Effect of Extraction Temperature

Temperature is another parameter that may have an effect on extraction efficiency. In order to examine this factor, extraction procedures were done in the range of 25–40 °C.

The results obtained from these tests showed that by increasing the temperature, the efficiency of the extraction process decreased because at high temperature the solubility of the extraction solvent in aqueous solution increases and part of the extraction solvent evaporates during stirring, which greatly reduces the volume of extraction solvent.

Therefore, it was found that an ambient temperature (25 °C) is the most suitable one for performing the extraction procedure.

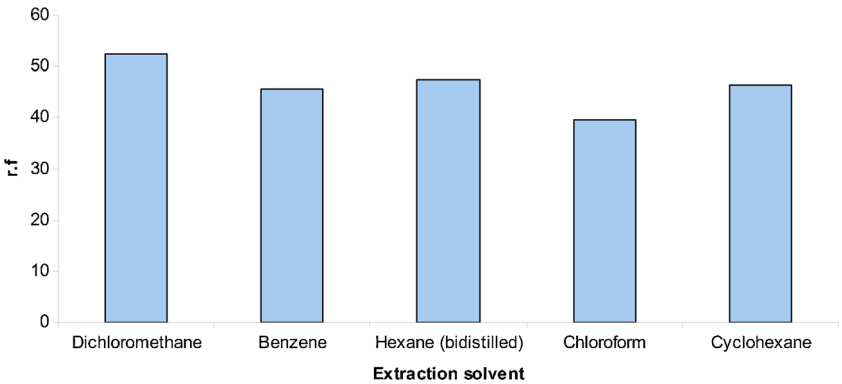

Fig. 4 The effect of the organic solvent type on the extraction efficiency. Conditions: sample volume, 10.0 mL; extraction temperature, 25 °C; extraction solvent volume, 3 mL; stirring rate, 1,500 rpm; extraction time, 5 min, and without salt addition

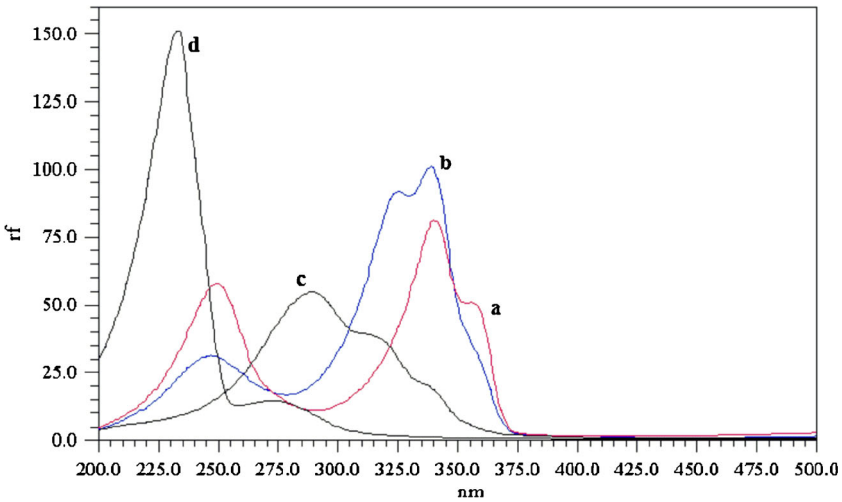

Fig. 5 Effect of the constant wavelength difference Δλ on the synchronous spectra of anthracene **a** 30, **b** 50, **c** 100, **d** 165 nm

Effect of Ionic Strength

To investigate the influence of ionic strength on our novel extraction procedure, various experiments were performed by adding different amounts of NaCl (0–5 %, w/v) to the aqueous solution before extraction with keeping the other parameters constant, where, no significant change in extraction efficiency was found.

Although NaCl is known for its salting-out effect in extraction procedures, but here it seems that has no significant effect on increasing the efficiency of the extraction process, thus no NaCl was needed in the extraction process to simplify the procedure.

Adjustment the Instrumental Parameters for Synchronous-Derivative Scanning

Since, the adjustment of instrumental parameters is very important to obtain symmetric spectrum shape with a narrow bandwidth, so different Δλ have been tested at 5 nm intervals and each time the synchronous spectrum has been plotted.

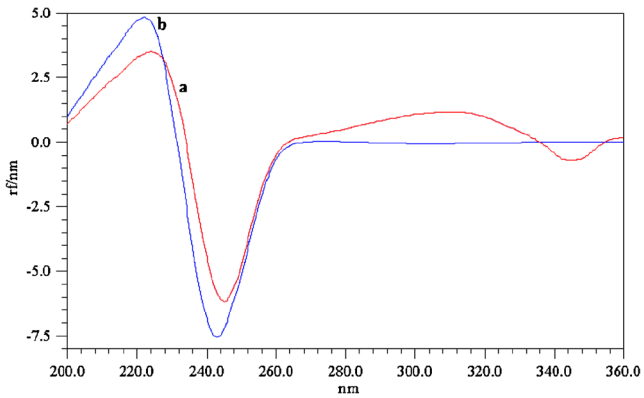

Fig. 6 First-derivative of fluorescence excitation spectrum (**a**) and of synchronous spectrum (**b**) of 100 ng mL^{-1} anthracene showing the improvement of response obtained by synchronous spectrum

The change in Δλ leads to a considerable change in the spectrum shape and the best synchronous spectrum was obtained for Δλ=165 nm which is slightly above stokes's shift (157 nm), (Fig. 5). Consequently, this Δλ was chosen for performing the synchronous scans of anthracene.

According to C. Cruces Blanco et al. [10], the band-narrowing effect obtained by the synchronous spectrum and the direct relationship between analyte concentration and the slope of the spectra obtained by the derivative technique, allows the synchronous-derivative approach to enhance the sensitivity of the analytical method which is very important in environmental analysis (Fig. 6).

Also, other instrumental parameters were adjusted in order to obtain the best shape and response, including the voltage of the photomultiplier, the scan speed and the response time [(400 V), (300 nm min^{-1}) and (8 s) were chosen].

Table 1 Validation parameters of the proposed spectrofluorimetric method for anthracene

Parameters	Assay result
Concentration Range (ng mL^{-1})	0.5–100
Correlation Coefficient	0.9995
Slope	0.0811
Intercept	0.0297
LOD (ng mL^{-1})	0.1
LOQ (ng mL^{-1})	0.3
$S_{y/x}$	0.08
S_a	0.03711
S_b	0.00079
Accuracy (Mean Recovery ± %RSD)	101.07±2.18
%Er	0.65

LOD limit of detection, *LOQ* limit of quantification, $S_{y/x}$ standard deviation of the residuals, S_a standard deviation of the intercept, S_b standard deviation of the slope, *%RSD*, relative standard deviation (%RSD = SD × 100/X where SD is the standard deviation and X is the mean recovery), *%Er* percent error (%Er = RSD/√n where n is the number of values)

Table 2 Repeatability (intra-assay precision)

Amount added (ng mL^{-1})	Amount found[a] (ng mL^{-1})	Recovery %±SD	RSD%	Er%
10	10.37	103.74±0.33	0.31	0.18
50	49.56	99.11±0.28	0.29	0.17
100	101.85	101.85±1.18	1.16	0.67

[a] Each result is the average of three separate experiments

The first-derivative graph of the synchronous spectra was calculated and the response was recorded at a wavelength of 244 nm from the peak to the baseline, which is proportional to the anthracene concentration and this was utilized for construction of the calibration graph.

Analytical Characteristics

By applying the previously mentioned conditions, the calibration graph was plot obtaining the regression equation y= 0.0811x+0.0297 (Table 1).

The limit of detection (LOD) was determined according to ICH Q2(R1) recommendations [29], by analyzing an appropriate number of samples near the detection limit and calculating the standard deviation of y-intercepts of the obtained regression lines (σ) followed by using the equation LOD=3.3 σ/S, where S is the slope of the calibration curve.

The results of inter-day and intra-day precision of the method were obtained by using 10, 50 and 100 ng mL^{-1} concentration of anthracene. The precision is acceptable due to the low values of SD and RSD%, respectively.

Also, the low values of Er% indicate a good inter-day and intra-day accuracy when using the proposed method, Tables 2 and 3.

The results of the proposed method for determination of anthracene were compared with a published method [30] and the statistical comparison between the results was performed using the student's *t*-test and F ratio at 95 % confidence level, as shown in Table 4.

The proposed method was found to be accurate and precise since there was no significant difference between the proposed method and the published one.

Table 3 Intermediate precision (inter-day assay)

Amount added (ng mL^{-1})	Amount found[a] (ng mL^{-1})	Recovery %±SD	RSD%	Er%
10	10.27	102.71±1.14	1.12	0.64
50	49.92	99.84±1.00	1.01	0.58
100	101.85	101.85±1.18	1.16	0.67

[a] Each result is the average of three separate experiments

Table 4 Statistical comparison between the proposed method and a published method

Values	Proposed method	Published method
Mean	101.07	100.00
Standard deviation	2.18	1.00
Variance	4.77	1.00
N	11	5
F	4.75 (5.96)[a]	
Student's *t* test	1.030 (2.145)[a]	

[a] Figures in parenthesis are the corresponding theoretical values for F and t at the 95 % confidence level

Applications

The proposed method was successfully applied for rapid determination of anthracene in tap water and river water (Nile water), by analyzing spiked water samples, as shown in Table 5.

It was also successfully applied for analysis of a contaminated environmental water samples (shisha water and industrial effluents) as shown in Table 5.

Conclusion

The results obtained from the present study show that, using of dispersive liquid-liquid extraction together with first-

Table 5 Results obtained from the analysis of real and polluted water samples

Sample	Added (ng mL^{-1})	Found[a] (ng mL^{-1})	Relative recovery %	RSD % (*n*=3)
Tap water	0	ND[b]	–	–
	2.00	1.91	95.54	2.27
	7.50	6.58	87.87	1.43
	14.40	14.68	102.00	2.66
Nile water	0	ND[b]	–	–
	7.50	6.39	85.40	0.68
	14.40	15.55	108.02	0.45
Shisha water (Water pipe)	0	86.27	–	–
Industial effluents of Kafr-El-Zayat Factory in El-Gharbiah	0	0.14	–	–
Industial effluents of Egyptian Textile Company in Alexandria	0	0.35	–	–

[a] Each result is the average of three separate experiments

[b] Not detected

derivative synchronous spectrofluorimetry for determination of anthracene in aqueous samples is a new simple, sensitive and time saving method for routine environmental analysis of anthracene with good sensitivity and precision. At the same time the proposed modification of dispersive liquid-liquid microextraction offers both flexibility in choosing an appropriate extraction solvent and a good extension for the use of such simple and time saving extraction procedures with the highly sensitive spectrofluorimetric techniques.

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