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RESEARCH ARTICLE

HPLC and spectrofluorimetric determination of pyrene in the Lebanese coast

Jamil Rima[*]

Laboratory of Physical Chemistry of Environmental Engineering and Biology, Faculty of Science II, P.O. Box 26110217, Lebanese University, Fanar, LEBANON

[*]**Corresponding author:** jamil.rima1@gmail.com

Abstract

In this article, homogeneous liquid-liquid extraction (HOLLE), combined with HPLC-fluorescence detector (HPLC-FLD and Fluorescence Spectrophotometer, has been developed for the extraction and determination of polycyclic aromatic hydrocarbons (PAHs) in polluted seawater. A liquid-liquid extraction followed by evaporative concentration method was used to determine the concentration of polycyclic aromatic hydrocarbons (PAH) in Lebanese marine water. Hydrocarbons were extracted from 450 ml of water into 75 ml n-hexane and then concentrated into 1 or 2 ml solution by evaporation. Internal standard addition method was used to determine the pyrene. Under the optimum conditions, repeatability was carried out by spiking pyrene at concentration level of 114.5 µg/l. the RSDs varied between 1.1 and 8.5% (n = 3). The LODs, based on S/N of 3, ranged from 0.025 to 0.25 µg/l. The pyrene determined in marine water was generated from the incomplete combustion of fuel oil in the powerplant of Zouk Mikael (Lebanon). Several PAHs were observed but the pyrene was easily indentified and quantified, due to the relative high concentration of this compound.

Key words: Pyrene, seawater, determination, HPLC, spectrofluorimeter, internal standard addition.

Introduction

Marine pollution in coastal areas is a subject of global interest, due to the large number of toxic substances transported from human activities (Filipkowska *et al.* 2005; Vagi *et al.* 2005; Lors *et al.* 2009). Among a long list of various contaminants, polycyclic aromatic hydrocarbons (PAHs) constitute a major environmental concern on marine ecosystems because of their adverse health effects on organisms, including endocrine disrupting activity (Kanaki *et al.*

2007; Zaghden *et al.* 2007). The release of PAHs in the marine environment is performed via processes such as the combustion of organic matter (pyrolytic origin). The naturally formed PAHs are biosynthesis products or come from oil upwelling and occur in the marine sediments at very low levels ranging from 0.01 to 1 ng g^{-1} dry weight. On the contrary, human activities are sources of a number of PAHs in the aqueous environment with the highest values being recorded in estuaries and coastal areas, as well as in areas with intense vessels transport and oil treatment (Shariati *et al.* 2003).

PAHs released into coastal seawaters have proven to be a prevalent problem and are thought to be responsible for the biological effects of marine life and some human's such as chromosomal problems in fish and other marine organisms. Polycyclic aromatic hydrocarbons (PAHs were detected in the surface seawater (Wolska *et al.* 1999; El-Deeb and Emara 2005). The authors were found that the main observations obtained from this profile are the abundance of pyrene (112ng/l) which represents about 41.2% from the total. By comparing this analysis with Saudi Red Sea coastal water and Gulf of Oman, it appears that "pyrene" is the most predominant PAH in the three coastal areas, it represents about 41.0, 71.0 and 46.0% from total PAHs.

Various studies have shown that pyrene and other PAHs with four benzene rings are among the most predominant PAHs in bivalves (Obana *et al.* 1983; Varanasi *et al.* 1985; Wade *et al.* 1988; Kaag *et al.* 1997). Moreover, pyrene is considered as one of the 16 most toxic contaminants for the environment and the dominant PAHs in the marine environment (Giessing *et al.* 2003).

Since PAH compounds are carcinogenic, identification and determination of these compounds in environment are very important (Wise *et al.* 1993). For this reason, their detection and monitoring has become an important problem and this has led to the development of new analytical methods with improved selectivity and sensitivity (Brouwer *et al.* 1994; Kiss *et al.* 1996; Nirmaier *et al.* 1996; Berset *et al.* 1999; Doong *et al.* 2000; Pino *et al.* 2002; Barranco *et al.* 2003). Although their solubility in water is very low, concentrations in the µg/l level are commonly encountered in the environment. Since these compounds are considered toxic at this level, their presence needs to be monitored.

The most common techniques used to analysis PAHs are gas chromatography (GC) (Miėge *et al.* 1998; Lacorte *et al.* 2000) and high performance liquid chromatography (HPLC) (Kiss *et al.* 1996; Kishikawa *et al.* 2003).

Our objective in this work is to study the pollution of sea water surrounding the powerplant of Zouk Mikael (North of Beirut) where particles from the combustion of heavy fuel oil were released into the atmosphere and in seawater.

Since the pyrene is considered the predominant in the list of PAHs and presents a significant indicator for the global of hydrocarbons. A rapid, inexpensive and simple method was developed to determine the pollution of the marine site in question by measuring the Pyrene using HPLC and spectrofluorimetric method.

Materials and Methods

Apparatus

Fluorescence Spectrophotometer Model *F-7000*: *Hitachi*, equipped with a 150 W Xenon lamp and 1.00 cm quartz cells, was used for the fluorescence measurements. Both excitation and emission slits were adjusted to 5 nm.

The analytical chromatographic system consisted of an Agilent 1100 series G-1322A vacuum degasser, a G-1313A automatic sample injector a G-1311A quaternary pump, a G-1316A column thermostat, a G-1315B diode-array detector, a G-1321A scanning fluorescence detector (all Agilent Technologies, Palo Alto, USA), and a Hypersil Green PAH column with guard column, 5 μm, 250 mm × 3 mm i.d. (Thermo Electron Corporation, Runcorn, UK). The system was controlled by use of Chem Station for LC 3D software (Agilent Technologies) installed on an IBM PC-compatible Pentium computer. Mobile-phases for HPLC were doubly distilled water and acetonitrile. Separations were performed at room temperature with a constant flow rate of 1 ml/min. For detection of these compounds λ=254 nm was used.

Reagents

Hexane, chloroform, ethanol and methanol were supplied from Merck (Darmstadt, Germany). Phenanthrene, anthracene, and pyrene were obtained from Fluka (Buchs, Switzerland), all with a purity of more than 97 %. The stock solutions of these compounds at a concentration of 1.86 mg/1 were prepared in ethanol. These standards were kept in the dark at 4 °C. Working solutions used for direct injection were made up in methanol. Mobile phases used for HPLC were mixture of HPLC-grade acetonitrile and de-ionized doubly distilled water filtered by 0.45 μm filter.

Stock Solution and Samples

Standard Solution

In a 100 ml flask a small Crystal pyrene is dissolved in ethanol-water (50/50 v / v). The mixture is then stirred until complete dissolution. The solution was then filtered through PTFE syringe filter to remove the remains of the crystals. The concentration of pyrene was determined by UV absorption spectrum taking in account the absorption extinction coefficient at 336 nm $\varepsilon = 5.10^4\ M^{-1}.cm^{-1}$. The peak of the HPLC chromatogram corresponding to 18.6 micrograms per liter of solution.

Sampling

Samples were collected from four polluted sites at the Lebanese marine coast. These samples were collected into pre-cleaned amber glass bottles and immediately were transferred to Laboratory for analysis. To avoid analyte adsorption problems on surface of bottle and vessels, some acetonitrile (10 %) was added to the samples, filtered through 0.45 µm filter and were transferred to cartridge for pre-concentration.

Extraction Method

A liquid-liquid extraction followed by evaporative concentration method was used to determine the concentration of polycyclic aromatic hydrocarbons (PAH) in Lebanese marine water.

A measured volume of sample, approximately 450 ml, is serially extracted with 75ml of hexane using a separatory funnel. The hexane extract is dried and concentrated to a volume of 1 ml. A 3.0 ml of acetonitrile were added to the extract and concentrated to a final volume of 0.5 ml. The extract samples are then injected in the HPLC.

Results and Discussion

HPLC Chromatogram of Pyrene as a Standard Using UV Detection

Mobile-phases for HPLC separation of these compounds with UV detection were doubly distilled water and acetonitrile. The analysis performed with 10% of acetonitrile. Separations were performed at room temperature with a constant flow rate of 1 ml/min. For detection of these compounds λ=254 nm was used. Figure 1 shows typical chromatogram of pyrene standard solution.

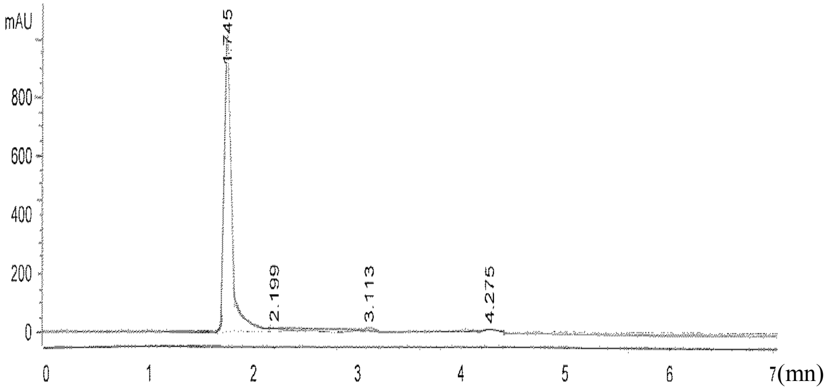

Figure 1. Chromatogram of pyrene standard by HPLC
Conditions: Mobile Phase; acetonitrile: water (10:90 V/V). Column; C18 (150×3.9 mm) 5 µm; Flow Rate: 1 ml/min; Wavelegth Detection; 254 nm; Pyrene=18.6 $\mu g ml^{-1}$

Identification of Pyrene in Seawater with HPLC Using UV Detection

After extraction of the PAH's from seawater using liquid-liquid extraction, 50 µl were withdrawn and injected in the HPLC. Figure 2 shows chromatogram of PAHs at λ=254 nm.

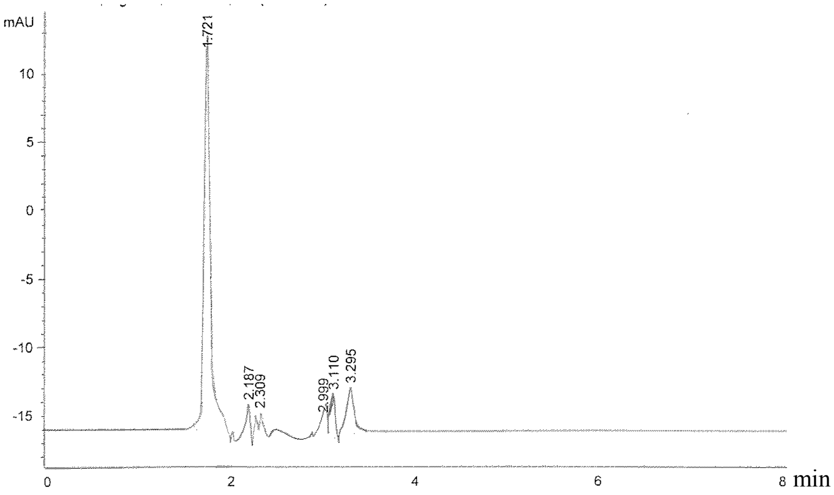

Figure 2. Chromatogram of seawater extract of sample collected from the Zouk Mikael powerplant area,Lebanon

By comparing the chromatogram of pyrene standard with that of the sample extracted from the seawater (Zouk Mikael), it can be observed that the pyrene, which appears at the retention time t = 1.74 minutes in the standard solution, appears also in the sample to be analyzed. Other small peaks were recorded in the chromatogram of samples assigned to other unidentified PAH's.

Identification of Pyrene in Seawater by Fluorescence Using a Fluorescence Spectrophotometer Model F-7000: Hitachi

For the Fluorescence Spectrophotometer, PAHs were extracted from seawater using hexane as solvent and separation funnel as apparatus. The extract was passed throughout anhydrous sodium sulphate followed by florsil column. Then PAHs were extracted by separation funnel containing 90 ml hexane and 30 ml water. PAHs fluorescence spectra were recorded by Spectrophotometer Model *F-7000*: *Hitachi* using a quartz cells.

Figure 3 presents the fluorescence spectrum of the extract taken from seawater in front of the powerplant of Zouk Mikael (Lebanon).

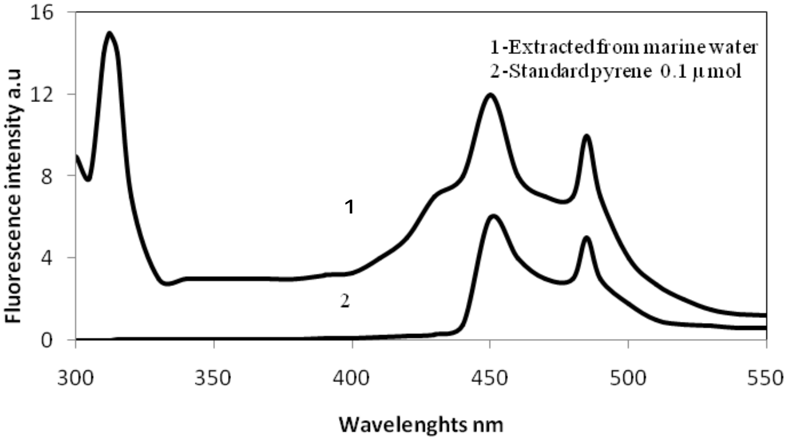

Figure 3. Fluorescence spectrum registered for the marine seawater of powerplant area of Zouk Mikael Lebanon (1);Fluorescence spectrum of pyrene standard (2)

The excitation wavelength is 280 nm and the emission spectrum starts from 300 to 500 nm. Between 300 nm and 350 nm, a broad band has been observed and attributed to emissions of PAH's with two and three cycles. In the region of 400 -500 nm, the fluorescence spectrum observed corresponds to PAH's with 4 or 5 cycles. By comparison with the fluorescence spectrum of pyrene, it appears that the fluorescence of pyrene is easily identifiable as illustrated in Figure 3.

As this figure shows the standard spectrum of pyrene (0.1 µmol) is superimposed exactly with that observed in the fluorescence spectrum of the seawater extract. Fortifying the samples of sea water by standard pyrene solution and studying the evolution of the spectra at 450 nm and 485 nm, it will be able to quantify the concentration of pyrene in the seawater using the internal standard addition method

Quantification of Pyrene in Seawater by Fluorescence Using the Internal Standard Addition Method

Calibration Curves and Recovery

Stock solution of pyrene was transferred into a volumetric flask to produce a solution with a concentration of 1µmol. The solution was shaken for 20-30 min until complete dissolution of the pyrene crystals. Samples for analysis were prepared by mixing 2 ml of the solution to be analyzed and different volumes of pyrene stock solutions (1-2-3-4 and 5 ml). Volumes of hexane were transferred to each sample to reach a final volume of 10 ml.

Concentration of Pyrene added varied from 0.1 µmol to 0.5 µmol Calibration curves were built for quantitative measurements Figure 4. Table 1 describes the preparation of the standard curve.

A spectrofluorimetric method using the internal standard addition was examined to quantitatively determine pyrene concentrations in samples. A calibration curve was described by the following equation:

$$IF^* = aC \text{ x } b$$

$$IF^* = \left(\frac{IF_0^*}{C_0}\right) \text{ x } C_{add} + IF_0^*$$

where IF* = (IF/IF0) normalized fluorescence intensity (arbitrary values) is equal to the ratio of the fluorescence intensity after adding the internal standard IF to the absorbance intensity before adding the internal standard (IF0), C0: solute concentration to be estimated. C0 is determined by the negative intercept of the curve with the abscissa axis (Muel and Lacroix 1960; Rima *et al.* 1982).

IF^*_0: Normalized fluorescence intensity of the starting solution, and Cadd: known added concentrations. The plot of IF* vs. Cadd is shown in Figure 4.

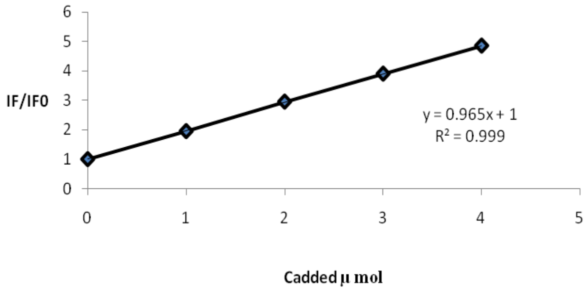

Figure 4. Calibration curve for the internal standard addition method

The internal standard used in this method was the pyrene that we would like to determine (C_0). To this initial solution, different known concentrations (Cadd) were added. The average recovery for five samples spiked with pyrene as described above in Table 1, was estimated to be 97% ±3.

Table 1. Composition of the samples used for pyrene standard analysis

Volume added of pyrene standard [1µmol] (ml)	Volume of sample to be analyzed (ml)	Volume of hexane (ml)
0	1	9
1	1	8
2	1	7
3	1	6
4	1	5
5	1	4

In Table 2 the validation parameters are summarized.

Table 2. Method validation parameters: Calibration curve for the internal standard addition method

Method	Spectrofluorimetric
Standard linearity intercept	-1.036
Slop	0.965
R^2	0.999
Range (µmol)	0.1 – 0.5
Precision (n=5) (RSD) (%)	3
Mean recovery(%) ± standard deviation	97
Detection limit µg/liter or ng/ml	0.018

Application of the Method on the Determination of Pyrene in the Marine Water of the Electric Central of Zouk Mikael-Lebanon

To determine the concentration of pyrene in sea water we will follow the method of internal standard additions Samples for analysis were prepared by mixing 2 ml of the extract obtained by liquid-liquid extraction realized in seawater, different volumes of pyrene stock solutions (1-2-3-4 and 5 ml) n-hexane was transferred to each sample to reach a final volume of 10 ml Table 3 contains the details of the composition of solutions to be tested.

Table 3. Composition of the samples used pyrene analysis in seawater using internal standard addition method

Volume of seawater extract (ml)	Volume pyrene stock solution [1µmol] (ml)	Volume of n-hexane (ml)
2	0	8
2	1	7
2	2	6
2	3	5
2	4	4
2	5	3

Figure 5 shows the internal standard addition curve for the pyrene determination in seawater of the Powerplant Zouk Mikael Lebanon.According to this curve the amount of pyrene was estimated by 114.5 µg/ liter.

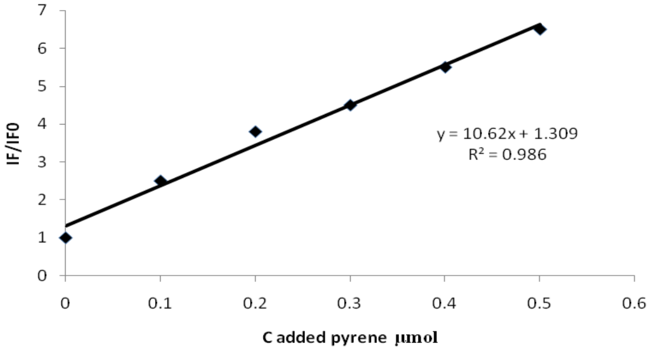

Figure 5. Internal standard curve for pyrene determination in seawater collected in Zouk Mikael powerplant area

Others PAHs should be in the samples taken from seawater but their quantities are relatively small compared to the pyrene concentration and therefore their emissions were not observed specifically for each of them. The recorded fluorescence spectra in the region 450 nm and 485 nm showed a background with the pyrene characteristic emission.

Conclusion

In this work we tried to assess the pollution of some sites on the Lebanese coast in particular sites polluted by incomplete combustion of heavy fuel oil in Zouk powerplant.

Using a simple rapid and inexpensive method, we could determine the concentration of pyrene in marine waters as a significant pollutant in the incomplete combustion of heavy fuel. This method is based on liquid-liquid extraction using hexane as a solvent for extraction of PAH's from seawater. The measurements were performed by two techniques. The first one is an HPLC equipped with a UV detector. The second technique is a spectrofluorometer Hitach-7000 for the determination of the fluorescence of PAH’s. The close results of the fluorescence with those obtained by HPLC, confirm the validity of the method. Internal standard method was used for the quantitative determination in the fluorescence measurements. It has been observed that the pyrene was among the majority's of PAHs obtained during the combustion of heavy fuel in the eletctrical powerplant of Zouk Mikael-Lebanon and the its concentration in the marine water was estimated to 114.5µg/liter.

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