Stability Studies of Selected Polycyclic Aromatic Hydrocarbons in Different Organic Solvents and Identification of Their Transformation Products

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Abstract

One of the problems in an HPLC laboratory is the preservation of samples and extracts prior to analysis without any changes. It has been found that degradation processes cannot be eliminated entirely, but they can be slowed down considerably. Photodegradation kinetics of fluorene, anthracene and benzo(a)pyrene were studied in various organic solvents. Solvents known as good media to store PAHs for a long time were selected with respect to avoid degradation. In the case of fluorene, 9-fluorenone and 9-hydroxyfluorene were detected as main photoproducts. Formation of anthraquinone and 1,8-dihydroxyanthraquinone during the degradation of anthracene was observed. Benzo(a)pyrene-4,5-dihydrodiol and one of the isomers of hydroxy-BaP-dione as products of benzo(a)pyrene photodegradation have been identified.

Keywords: polycyclic aromatic hydrocarbons, photodegradation, degradation products, sample stability

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants originating from natural and anthropogenic pyrolysis of organic matter such as forest fires, automobile exhaust, coal and oil refining processes. Their abundance and persistence in several polluted environmental compartments such as sediments [1], surface waters [2], airborne particulate matter [3] and drinking water [4] have been reported.

PAHs are hydrophobic compounds and their persistence in the environment is chiefly due to their low water solubility. Generally, PAH solubility decreases and hydrophobicity increases with an increase in the number of fused benzene rings [5]. Most polycyclic aromatic hydrocarbons, especially the highly condensed compounds with 4 or more rings and their metabolites, have a variety of mutagenic and carcinogenic effects on microorganisms, plants and animals, and are classified as compounds with significant human health risks [6].

In the environment, primary removal processes of low molecular weight PAHs are microbial degradation and evaporation. Higher molecular weight aromatics are less water soluble, which makes biodegradation difficult. Thus, these compounds are effected by photochemical oxidation. This process is known for PAH solutions, PAHs in pure solid form or adsorbed onto solid substances. Exposure of aromatic compounds to light produces partially oxidized intermediates which are more susceptible to biodegradation than parent compounds. Thus, photodegradation has been suggested as pre-treatment strategy for biodegradation [7].

Many studies have been performed on the photolysis of individual PAHs in natural waters under irradiation. For example, Zepp and Schlotzhauer [8] found that the photolysis rates of selected PAHs in a natural water body were

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quite fast and the photolysis half-lives of PAHs ranged from several minutes to several hours. Mill et al. [9] found out that the photolysis half-lives for benzo(a)pyrene and benzo(a)anthracene in water under sunlight irradiation were 0.69 and 5.0h, respectively. However, relatively little is known of photodegradation of PAHs when they are not irradiated and present in pure solvents. Only some studies have demonstrated that PAHs undergo fairly rapid transformations when exposed to light in organic solvents and organic solvent-water mixtures [7, 9-12]. Quite a few researchers studied photochemical behaviour of PAHs in particular matters and aerosols [13], pine needles [14].

Knowledge of degradation processes is essential from the analytical point of view and related to it reliability of the obtained results, because it can allow it to reduce or even eliminate possible errors associated with storage of samples of extracts and standard solutions to be used in the final determination.

The main aim of this work was to recognize the influence of selected factors on stability of fluorene, anthracene and benzo(a)pyrene in different solvent solutions. The dependence of photodegradation rate of selected PAHs on the effect of primarily light photodegradation, and also on the type of solvent has been investigated. Main photodegradation products were identified.

Experimental Procedures

Chemicals

Methanol, acetonitrile, hexane of HPLC-gradient grade was obtained from Merck (Darmstadt, Germany), ultrapure water was provided by Milli-Q water purification system (Millipore, Bedford, MA, USA). Dichloromethane, dimethyl sulfoxide (DMSO) and cyclohexane (purity > 99.5) were also obtained from Merck.

The following standards: fluorene (FL), anthracene (ANT) and benzo(a)pyrene (BaP) were obtained from Sigma – Aldrich (Seelze, Germany). All the standards had a minimum purity of 99%. Stock solutions of the standards were prepared at the concentration of 1 mg ml^{-1} (weight/volume). For quantitation the calibration curves

were prepared basing the solutions containing analytes at concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5 µg ml^{-1}.

Borosilicate glassware (Merck) used for experiments was washed in chromic acid cleaning mixture for 12h, then rinsed with deionized water, methanol, and acetone and dried at 110°C for 3h.

PAH-Stock-Solutions

A set of dilution of analyte solution at the concentration of 5 µg l^{-1} was made in the following solvents: methanol, acetonitrile, DMSO, hexane, cyclohexane and dichloromethane.

PAH standard solutions, prepared in the above solvents, were stored at room temperature (20 ± 2°C) with a full sunlight exposure in the laboratory (i.e. in glass containers fully transparent to the light). It is well known, that the sunlight reaching the Earth has wavelength of $\lambda \geq 290$ nm. Because of their aromatic structure, PAHs strongly absorb ultraviolet light in the UV-A ($\lambda = 320$–400 nm) and UV-B ($\lambda = 290$–320 nm) range of natural sunlight [15].

Sample Preparation

Solutions prepared in methanol and acetonitrile were analyzed directly, while solutions in DMSO were diluted with de-mineralized water at a 1:1 ratio before injection. In the case of solutions in hexane, cyclohexane and dichloromethane, solvent was completely evaporated under a gentle stream of nitrogen; the remaining solid was dissolved in methanol (volume equal to the volume of the original solvent). The solutions prepared in this way were injected onto an HPLC column. The investigations were carried out for a series of standard solutions containing analytes at different concentration levels (i.e. 0.1 µg ml^{-1}; 1 µg ml^{-1} and 5 µg ml^{-1}). The studies revealed that the analyte level had no effect on the percent decrease of the compound during evaporation of the solvent.

The average recovery of analytes (after evaporation to dryness) for each liquid media and corresponding relative standard deviations RSD (n = 7) are presented in Table 1.

Table 1. The average recovery of analytes and relatively standard deviations RSD (n = 7).

Organic solvents	FL		ANT		BaP	
	Recovery [%]	RSD [%]	Recovery [%]	RSD [%]	Recovery [%]	RSD [%]
Methanol	92 ± 0.1	0.3	96 ± 0.1	0.3	89 ± 0.1	0.3
Acetonitrile	96 ± 0.1	0.2	95 ± 0.1	0.2	91 ± 0.1	0.3
DMSO	89 ± 0.2	0.3	91 ± 0.1	0.3	90 ± 0.2	0.3
Dichloromethane	70 ± 9.9	20.1	93 ± 4.1	6.2	80 ± 6.3	8.1
Hexane	69 ± 5.5	13.9	90 ± 1.5	2.4	84 ± 5.8	7.3
Cyclohexane	71 ± 5.2	11.0	90 ± 2.1	3.4	86 ± 6.7	9.2

Table 2. Chromatographic conditions used for determination of fluorene, anthracene and benzo(a)pyrene and their degradation products.

Parameters	Details		
Column	LiChrospher RP-18e (5μm, 125 x 4mm I.D)		
Mobile phase	(A) H_2O (B) acetonitrile/methanol (1:1 v/v)		
Flow rate	0.8 ml min^{-1}		
Temperature	25°C		
Injection volume	20 μl		
UV detection	250 nm, 260 nm, 295nm		
Gradient	Time [min]	% A	% B
	0.00	25	75
	10.00	0	100
	15.00	0	100

Table 3. Chromatographic conditions used for determination and identification of benzo(a)pyrene transformation products.

Parameters	Details		
Column	LiChrospher RP-18e (5μm, 125 x 4mm I.D)		
Mobile phase	(A) H_2O (B) methanol		
Flow rate	1 ml min^{-1}		
Temperature	25°C		
Injection volume	20 μl		
UV detection	254 nm		
MS detection	APCI interface, positive ionisation mode with 120eV fragmentor voltage, a 450°C vaporizer temperature, 350°C drying gas temperatures, 50 psig (1psi = 6894.76 Pa) drying gas pressure, 6 l min^{-1} drying gas flow rate, 6μA corona current and capillary voltage 4000V		
Gradient	Time [min]	% A	% B
	0.00	20	80
	4.00	20	80
	6.00	18	82
	9.00	8	92
	10.00	0	100
	15.00	0	100

High relative standard deviation obtained for the determination of fluorene (in case of standard solutions prepared in dichloromethane, hexane and cy-clohexane) are closely related to the vapour pressure of this compound (0.72 Pa at 20°C [16]). The higher the volatility of an analyte mean the greater the losses during its evaporation (lower recovery), and hence the lower the precision of determinations. Extraordinary high RSD in dichloromethane has been observed for FL; dichloromethane is the most volatile solvent, while FL has the lowest molecular weight (MW for fluorene is 166, while for ANT and BP it is 178 and 252, respectively); under such conditions the highest RSD has been obtained.

Analytical Methods

The chromatographic experiments were performed using a high performance liquid chromatograph (Agilent 1100 Series) equipped with a diode array detector, mass spectrometric detector, and a column oven, binary pump and autosampler. Detailed chromatographic conditions are presented in Tables 2 and 3. Quantitation limits for fluorene, anthracene and benzo(a)pyrene were 0.03; 0.09 and 0.07 μg ml^{-1}, respectively.

Kinetic Calculations

Kinetics of PAH degradation was calculated using first-order equation:

$$\ln C/C_0 = -kt \qquad (1)$$

where C_0 and C are the compound concentration at time zero and t, respectively, and k is the rate constant.

The results were plotted using the natural logarithm of the compound concentration as a function of time. First-order degradation rate constants were determined by regression analysis. Half-lives $t_{1/2}$ were calculated using Eq. (2) which was derived from Eq. (1) by replacing C with $C_0/2$:

$$t_{1/2} = \ln2/k \qquad (2)$$

In some reactions the rate is independent of the concentration of the reactant or reactants, and these are termed zero-order reactions. Photochemical reactions, in which the rate-determining factor is the light intensity, rather than the concentration of the reactant, may be zero-order reactions. In this case, kinetics of PAH degradation was calculated using zero-order equation:

$$C = -kt \qquad (3)$$

Half-lives $t_{1/2}$ were calculated using the following equation:

$$t_{1/2} = C/(2k) \qquad (4)$$

Results and Discussion

Stability in Organic Solvents

Relatively little is known of photochemical reactivity of PAHs when they are present in organic solvents. It is generally accepted that photodegradation of PAHs in solutions is an oxidative process which is highly accelerated by the presence of photo-initiators. In general, the more polar the solvent is, the faster the degradation process of PAH [7]. The rate of the photodegradation process is also affected by the amount of dissolved oxygen, temperature and light intensity.

Degradation of FL, ANT and BaP in selected organic solvents was monitored for more than 3 months. Fig. 1 presents the dependence of concentration of non-degraded analytes on the type of organic solvent.

For the first 20 days, the concentration of FL, ANT and BaP remained constant in all examined solvents, with the exception of ANT and BaP stored in DMSO. In both cases, the process of degradation of these compounds in DMSO was very fast and after only six days the amount
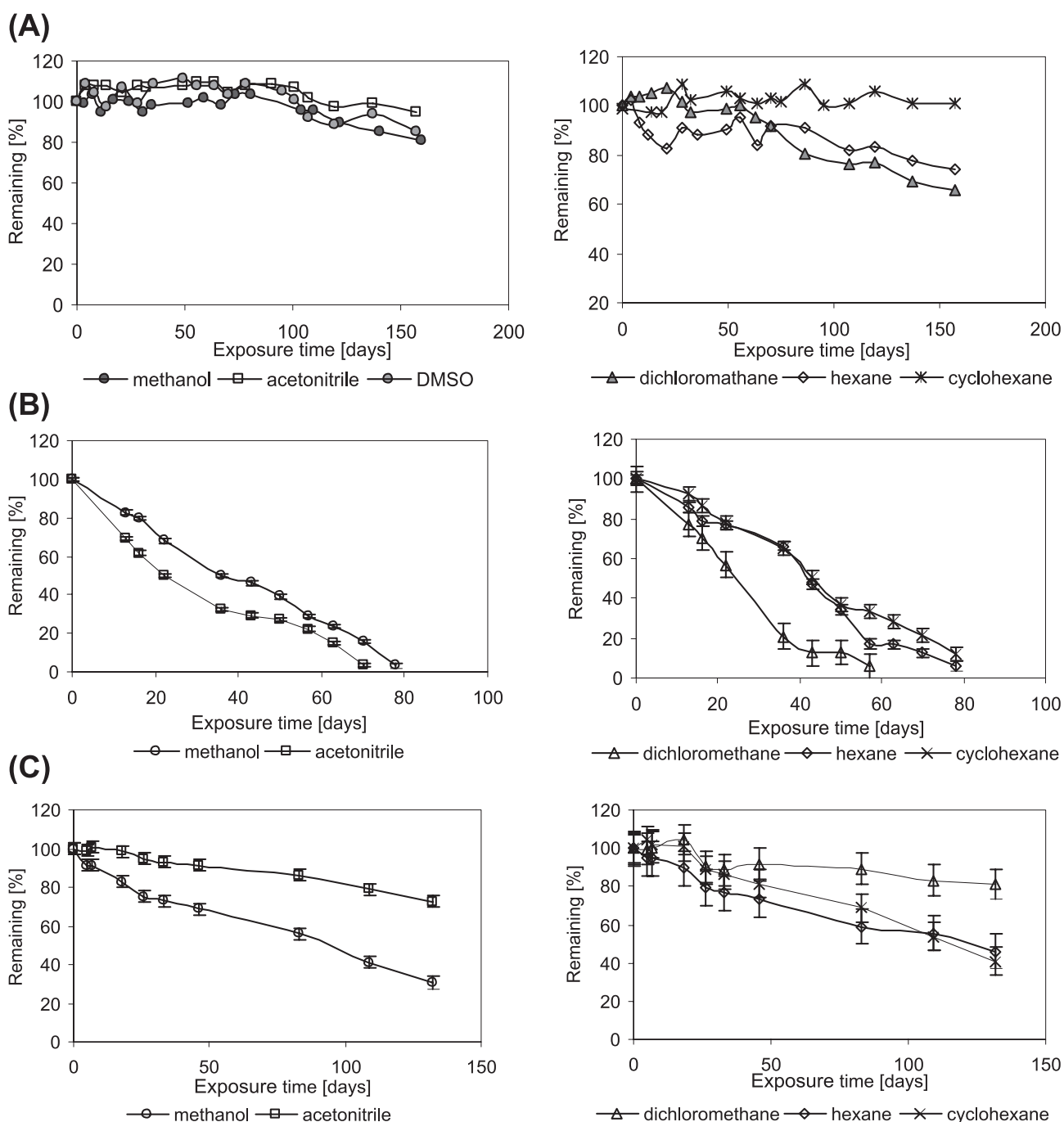
(A)

(B)

(C)

Fig. 1. Graphical presentation of process of degradation of PAHs in organic solvent: (A) fluorene; (B) anthracene and (C) benzo(a) pyrene.

of analytes in a sample was below the detection limit of the analytical procedure used. Due to the fast degradation of both anthracene and benzo(a)pyrene stored in DMSO, additional studies were carried out (monitoring every few hours), which enabled subsequent determination of basic kinetic parameters: rate constant of the reaction and half-lives of these analytes. The effect of storage time of standard solutions of anthracene and benzo(a)pyrene in DMSO is shown in Fig. 2.

The increased rate of photolysis of ANT and BaP results from the fact that DMSO is a solvent having strong oxidizing properties and in the presence of photons of light it becomes a source of free radicals which initiate further photochemical reactions.

After 80 days of exposure, anthracene was completely degraded in all examined solvents, 50% of BaP was degraded in methanol, while nearly 40% in hexane and cyclohexane. In the case of fluorene, only 40% loss was observed in dichloromethane solutions. The unusually high stability of FL in organic solvents can be explained, first of all, by the lack of radiation of wavelength absorbed by fluorene; it shows good absorption of the light in a wavelength range between 240 and 305 nm [10]. The measure-

ments could be made for solar irradiation whose energy is not sufficient to cause fast degradation. Possibly, the molecules of fluorene and solvent did not absorb enough energy for photodegradation (free radical formation through chain reaction) to be initialized. On the other hand, conditions used for the entire measurement in the laboratory were as close to "naturally" occurring as possible. The inconsistency of the results obtained with ANT and BaP and FL causes the stability of PAHs in organic solvents to be checked before long-term storing.

Solvent replacement prior to chromatographic analysis results in an increase in measurement error. As the plots illustrating sample storage in dichloromethane, hexane and cyclohexane indicate, a larger scattering of the results is observed than that for the solvents constituting the original sample matrix.

Rate constants and half-lives obtained for degradation of ANT and BaP in organic solvents are given in Table 4. It was impossible to determine kinetic parameters for the changes taking place for fluorene.

Fluorene Degradation Products

On the basis of agreement of retention times and comparison of the UV absorption spectra obtained for samples of standard solutions of a reference and fluorene it was concluded that the conversions taking place in solutions of fluorene in dichloromethane and hexane result in the formation of 9-fluorenone.

Chromatograms obtained during analysis of a standard solution of fluorene, with the parts of chromatograms showing extraneous (not observed before) peaks magnified, are shown in Fig. 3.

During attempted identification of trace amounts of compounds formed as a result of degradation of fluorene in dichloromethane and hexane, the presence of 9-hydroxyfluorene was established. Since identification of this compound based on comparison of its UV spectrum with the spectrum of the reference compound (9-hydroxyfluorene) was impossible due to an insufficient amount of the newly
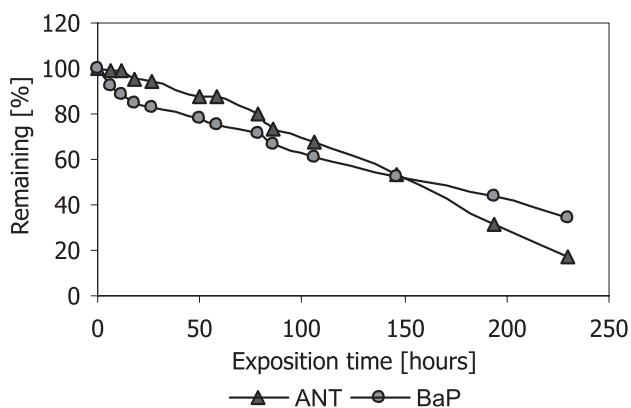

Fig. 2. Graphical presentation of dependence of process of degradation of anthracene and benzo(a)pyrene in DMSO model solutions.

Table 4. Anthracene and benzo(a)pyrene photodegaradation rate constants (k) and half-lives ($t_{1/2}$) for various organic solvents calculated on the basis of analytical data.

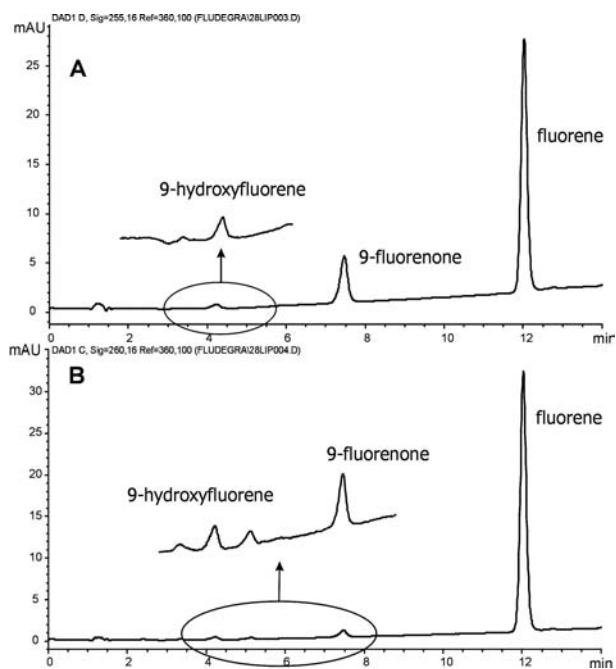| Organic solvents	ANT			BaP		
	R^2	k [concentration per day]	$t_{1/2}$ [days]	R^2	k [1 per day]	$t_{1/2}$ [days]
Methanol	0.995	0.0592	42	0.979	0.0081	86
Acetonitrile	0.936	0.0578	24	0.976	0.0023	301
DMSO	0.989	0.0086	140 [h]	0.975	0.0099	190 [h]
Dichloromethane	0.972	0.0881	26	0.786	0.0016	433
Hexane	0.968	0.0563	41	0.981	0.0055	126
Cyclohexane	0.988	0.0511	42	0.967	0.0066	105

Fig. 3. Chromatograms obtained during the analysis (HPLC-DAD) of sample of fluorene stored in: (A) dichloromethane and (B) hexane.
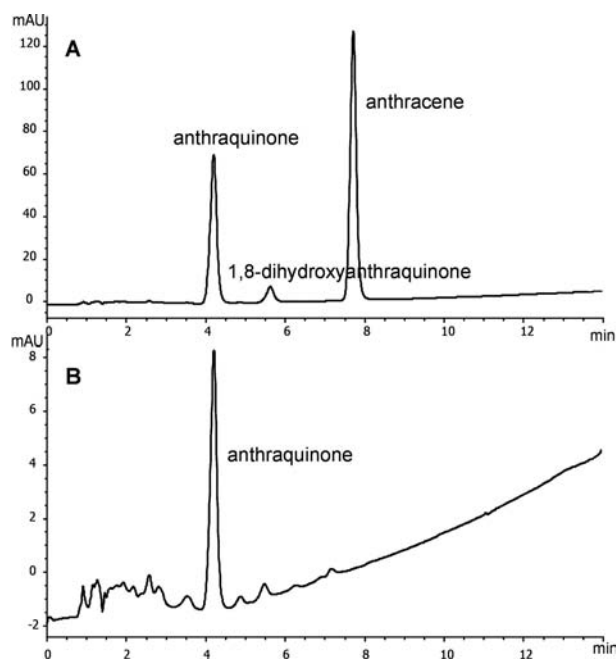Peaks: 1) 9-hydroxyfluorene; 2) 9-fluorenone; 3) fluorene.

Fig. 4. Chromatograms obtained during analysis of anthracene model solutions in methanol after (A) 36 days and (B) 75 days of sample exposure.
Peaks: 1) anthraquinone; 2) 1,8-dihydroxyanthraquinone; 3) anthracene.

formed compound, identification was carried by matching retention times of the compound formed through the degradation of fluorene with the reference (9-hydroxyfluorene). For confirmation purposes, several analyses of samples in which the presence of 9-hydroxyfluorene was found and reference solutions were performed, while varying chromatographic conditions (the composition of mobile phase). In all cases, the agreement of retention times of the compound identified as 9-hydroxyfluorene with the reference was observed.

Anthracene Degradation Products

Anthracene turned out to be the compound which very quickly underwent photodegradation yielding anthraquinone as the main degradation product. It should be pointed out that the presence of anthraquinone established all the solvents in which anthracene was dissolved. According to the literature data [17], a substantial fraction of anthracene (19-25%) undergoes degradation via photochemical oxidation with the formation of anthraquinone as the main degradation product. Chromatograms of standard solutions of anthracene stored in methanol after 36 and 75 days of exposure are shown in Fig. 4.

It follows from the chromatograms shown that not only anthracene but also anthraquinone underwent degradation. Identification of the compounds formed during sample exposure (anthracene in methanol) was carried out by comparison of the UV spectra with those obtained for reference compounds. The UV spectra of anthraquinone

and 1,8-dihydroxyanthraquinone obtained during analysis of standard solutions of anthracene are shown in Fig. 5.

Benzo(a)pyrene Degradation Products

Photodegradation of BaP performed in different organic solvents fully exposed to the sunlight and in the presence of oxygen (conditions as close to the naturally occurring as possible) resulted in additional peaks observed on chromatograms after 2 months. HPLC analysis with DAD and mass spectrometry (MS) detection of the organic solution revealed a number of BaP degradation products as presented in Fig. 6.

In the case of compound 1 maximum absorbance in UV spectrum and MS spectrum pattern suggest that BaP-4,5-dihydrodiol has been detected. This metabolite was identical in its MS spectrum and UV spectrum with the reference BaP-4,5-dihydrodiol published elsewhere [18, 19]. The parent ion m/z 286 immediately undergoes changes [loss of $2H_2$] that give ion m/z 283, further loss of CO $[M – 28]^+$ results in ion m/z 255. Another two further protonated fragments are observed at ion m/z 241 and ion m/z 239, both probably derived from the protonated molecule and the molecule after preceding loss of hydrogen molecule (H_2). MS spectrum and reaction scheme for the compound 1 identified as BaP-4,5-dihydrodiol has been shown in Fig. 7.

Compound 2 has the base peak at m/z 299 and fragment ions at m/z 255 $[M – 44]^+$, m/z 267 $[M – 32]^+$ and m/z
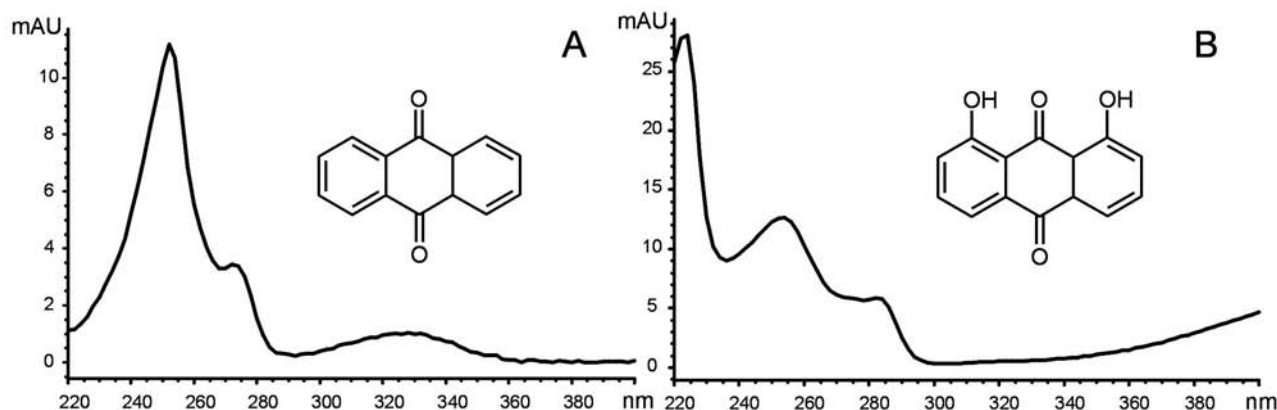
Fig. 5. Absorbance spectra and chemical structures of compounds identified as (A) anthraquinone and (B) 1,8-dihyroxyanthraquinone.
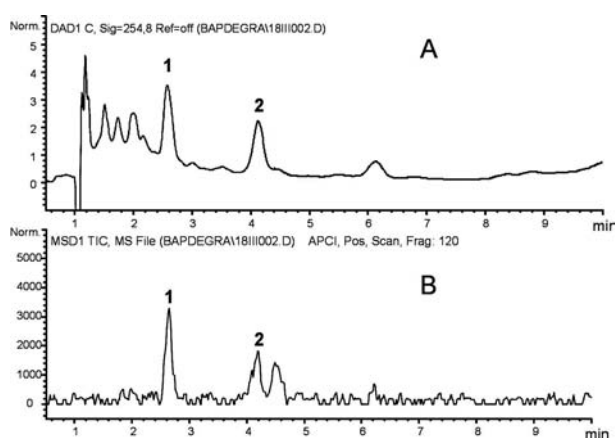

Fig. 6. HPLC-DAD-MS analysis chromatograms of BAP solution in cyclohexane after 2 months of exposition: (A) DAD response monitored at 254 nm; (B) APCI-MS (SCAN mode). *Peaks:* 1) BaP-4,5-dihydrodiol; 2) 2-hydroxy-BaP-1.6-dione.
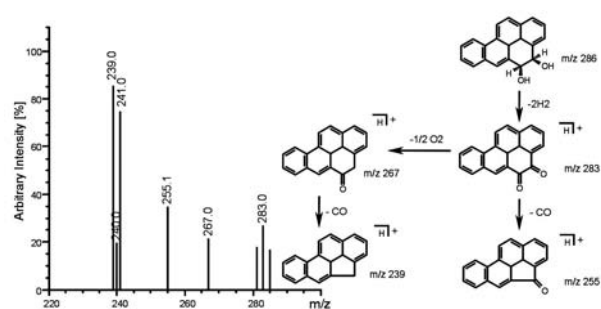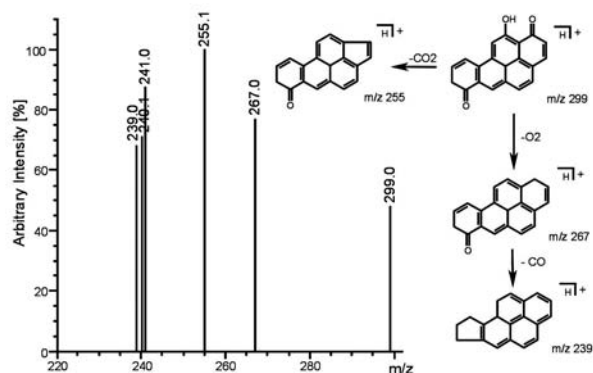

Fig. 8. Scheme of reaction leading to formation of 2-hydroxy-BaP-1.6-dione during the degradation of BaP and it mass spectrum.

served in case analyte was stored in differents solvents; however, the time of their occurence was different.

Conclusions

Photodegradation and biodegradation are major degradation processes which can naturaly clean up the environment. Photodegradation of PAHs, which is assumed to be a preliminary process after which the microbial decomposition of compounds occurs, causes the formation of partially oxidized, intermediate compounds that are more susceptible to biodegradation than the parent compounds. Stability of fluorene, anthracene and benzo(a) pyrene in different solvent solutions has been studied. The dependence of photodegradation rate of selected PAHs on the effect of primarily light photodegradation and the type of solvent has been mknitored over a period of three months. The increased rate of photolysis of antracene and benzo(a)pyrene was observed in DMSO (in comparison with methanol, acetonitrile, hexane, cyclohexane and dichloromethane), which results from the fact that DMSO is a solvent having strong oxidizing properties; thus in the presence of photons of light it becomes a source of

Fig. 7. Scheme of reaction leading to formation of BaP-4,5-dihydrodiol during the degradation of BaP and it mass spectrum.

241 $[M - 28]^+$, representing probable losses of CO_2, 2O and CO, respectively. Fig. 8 shows the suggested structure and MS fragmentation for the second metabolite, which can be identified as 2-hydroxy-BaP-1.6-dione ($C_{20}H_{10}O_3$; M=298 g mol^{-1}). In addition, other isomers are probably formed. Transformation products of BaP have been ob-

free radicals which initiate further photochemical reactions. Remarkable stability of fluorene in organic solvents has been observed, which can be connected with either the lack of radiation of wavelength absorbed by fluorene (conditions for all measurements were as close to "naturally" occurring as possible) or solar irradiation was not sufficient to cause degradation within 200 days. Main photodegradation products were identified. For fluorene the occurence of 9-hydroxyfluorene has been observed. The presence of anthraquinone as main degradation product of antracene was established for all examined solvents. BaP-4,5-dihydrodiol and 2-hydroxy-BaP-1.6-dione has been detected in the case of degradation of benzo(a)pyrene. The results can be helpful in selecting appropriate conditions and allowable time for storing sample extracts for analysis of polycyclic aromatic hydrocarbons. Therefore, understanding the mechanism responsible for PAHs degradation is required.

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