===== **ANALYTICAL CHEMISTRY OF WATER** =====

Determination of Polycyclic Carbohydrates in Atmospheric Water by the Method of Chromatography

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Abstract—Isomers of benzoperene were studied. Calculated values of hydrophobicity of their molecules were analyzed. The search for optimal conditions of extraction removal from atmospheric water of isomers of benzopyrene and chromatographic separation of benzo(e)pyrene and benzo(a)pyrene in atmospheric water (snow) was made. The concentration of benzo(e)pyrene and benzo(a)pyrene in atmospheric water (snow) polluted by polycyclic aromatic hydrocarbons (benzo(a)anthracene, benzo(b)fluoranthene, chrysene) was determined.

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INTRODUCTION

Pollutants of technogenic origin contaminate the environment and, in the first place—the air and atmospheric water. Atmospheric precipitations (for instance, snow making the snow cover and protecting soil and winter crops against winterkilling adsorb and concentrate pollutants to the snowbreak. The known pollutant of the second class of danger benzo(a)pyrene owing to destructive effect on the environment and human organism attracted the attention of scientists more than two hundred years ago. At the same time, due to the absence of instrumental methods of control as well as information on the chemical composition made it possible to clarify the mechanism of getting into the environment of not only benzo(a)pyrene, but also other polycyclic aromatic hydrocarbons (PAH), whose main source is the processes of burning of organic materials of technogenic origin. Afterwards burning products of fuels and lubricants are sorbed by snow, which for a long time lies along the roads.

According to the requirements of GSanPiN 2.1.4-171-10 the concentration of benzo(a)pyrene should be determined after liquid–liquid extraction by the method of highly effective liquid chromatography with fluorescent detection only in drinking water of the first and high categories at the level of respective 0.005 and 0.001 μkg/dm^3. Standards of pollution by this carcinogen of surface and atmospheric waters was not established. Taking into account foreign experience of steady development of agriculture based on principles of "green economy" oriented on preservation and restoration of natural resources in the interests of survival of mankind there appears the necessity in rationing and laboratory control over carcinogens in particular isomers of benzopyrene.

The objective of the present research is determination of the most effective conditions of extraction and chromatographic analysis in atmospheric water of isomers of benzopyrene—benzo(e)pyrene and benzo(a)pyrene hereinafter (BeP and BaP) creation and approbation of techniques of laboratory control over the content of isomers of benzopyrene in atmospheric water (snow).

EXPERIMENTAL

The research uses model systems based on deionized water and samples of snow taken near roads and service stations and in rural areas at a distance of 50 m off the road. Snow samples were converted into melt water, which was investigated after thermostating (20°C). Solutions in acetonitrile of analytical standards of individual chemical compounds and mixtures of polycyclic aromatic hydrocarbons manufactured by Sigma-Aldrich and reference—material with the known content of these hydrocarbons were used. In the research solvents of qualification "for chromatography", "pure for analysis" as well as toluene, chloroform, isopropanole, aceto-

nitrile, deionized water were used plus reagents and sorbents for fulfilling the stage of preparing the sample by the method of solid phase extraction (SFE), SFE cartridges (Agilent ChromSep, RAH).

The concentration of isomers of bensopyrene was determined by the method of liquid chromatography with a fluorescent detector (LC/FLD) on the HPLC ULTIMate 3000×2 Dual pump device of the Dionex company fitted with columns SUPELCOSIL LC-PAH. In chromatographic control of PAH the mode of gradient elution was used. The first 20 min they were eluted by the mixture of acetonitrile : deionized water (4 : 1) at the velocity of the flow up to 0.4 cm^3/min; then during subsequent 5 min in an automatic mode the eluent was replaced by acetonitrile and increased the rate of the current to 1.0 cm^3/min. Acetonitrile was fed to the system for 30 min then at gradual decrease of the current rate from 1.0 to 0.4 cm^3/min for 5 min acetonitrile was replaced by a mixture acetonitrile : deionized water (4 : 1), which was used for 10 min for conditioning of the column. The separation of PAH mixtures on chromatographic columns was controlled by means of a fluorescent detector tuned to the measurement of the PAH signals at $\lambda_{exc} = 290$ nm and $\lambda_{emission} = 430$ nm.

RESULTS AND DISCUSSION

Benzopyrene in the environment is represented by two structural isomers—BaP (I) and BeP (II):
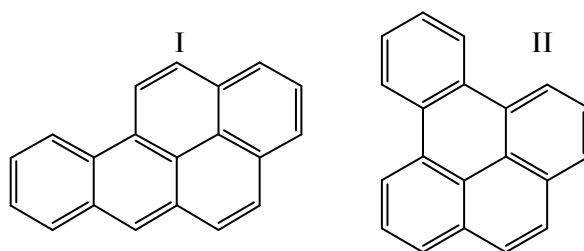
According to the data [1] they are high-boiling substances with low-pressure vapors and solubility in water. For BaP carcinogenic activity was established, while BeP is highly toxic, but is not carcinogenic [1, 2]. In [3] it is shown that toxicological and carcinogenic effects of individual substances is enlarged in their mixtures. This is an evidence that in the control of pollutants it is necessary to identify not only BaP, but also a substance which manifest a toxicological effect, reduce the protective function of an organism and enlarge carcinogenic activity of benzo(a)pyrene. Such substances may include its isomer benzo(e)pyrene. As is known, PAH are lipofilic substances however literary sources give parameters only for carcinogens [1−3]. Therefore in our paper for definition of distribution and maintaining isomers of benzopyrene in atmospheric water the parameter of hydrophobicity (low Pow) is calculated (Table 1).

Table 1. Literary and design data characterizing structural isomers of benzopyrene ($C_{20}H_{12}$)

Indicator		Benzo(a)pyrene or 3.4-benzopyrene, carcinogen	Benzo(e)pyrene or 4.5-benzopyrene, not a carcinogen
log Pow (statistical deviation) calculated by the method	Crippen [4]	5.34	5.34
		(0.47)	(0.47)
	Vishvanadhan [5]	5.37	5.37
		(0.49)	(0.49)
	Broto [6]	5.72	5.72
		(0.34)	(0.34)
T_{kip}, °C		496	492

As is seen from the specified table, the parameters of hydrophobicity of isomers of benzopyrene obtained within the framework of one calculation method coincide and slightly differ from calculation by different methods. The difference of calculated parameters logPow is based on differences of the pool of fragments of the molecular structure and possible ions of substances predetermined by authors of used methods [4−6]. The values obtained in three methods provide a possibility of prediction that binding of the carcinogen of benzo(a)pyrene and no the carcinogen of benzo(e)pyrene by atmospheric water and the system atmospheric water−condensate of fuel and lubricating materials will not be selective.

Since isomers of benzopyrene are highly-boiling their laboratory control is done by the methods of the reverse-phase highly effective liquid chromatography (HELC) [7] although for investigation of some PAH methods of gas–liquid or gas chromatography may be used [8].

Among known standardized method of PAH control [9–12] the information on the conditions of identification and quantitative measurement of benzo(e)pyrene in the mixture with benzo(a)pyrene in the literature was not found.

The PAH control techniques contain recommendations for the preparation of the samples of the research objects and methods of instrumental control of extracted analytical substances—carcinogen and PAH, which have a priority in laboratory control and whose number varies from 4 to 15 chemical compounds [13–14]. According the studied methods of sample preparation they lie in extraction removal of PAH from the matrix of the object by organic solvents using methods of liquid–liquid extraction or selective sorbents by the method of solid–phase extraction (SPE).

The measurement of the PAH content is conducted by the methods of chromatography whose main requirements is high sensitivity and selectivity. High selectivity of determining microquantity of BaP in mixtures with the priority laboratory control of PAH [12] is achieved when using HELC with fluorescent detector (HELC/MC/MS. The method of gas chromatography with mass-spectroscopy (GC/MS) does not allow one to separate not only structural isomers of benzopyrene, but their mixtures with normalized benzo(b)fluoranten (BbF) since due electronic ionization of BaP, Bep and BbF molecules fragments of molecules–ions with identical weight are created characterized by the parameter m/z: 252; 250; 253; 126; 113; 148.

The HELC method with fluorescent detection used for the analysis of mixtures of benzo(a)pyrene, benzo(b)fluoranten, chrisen and benzo(a)antracene in the control of food products ensures high selectivity and accuracy in measuring these substance [13, 14]. For identification of benzo(e)pyrene mixtures of benzo(e)pyrene with PAH were composed.

Figure 1 shows graduation relationships between the value of the peak area on the chromatogram and the concentration of analytical substances in the solution. In the regression analysis it was established that the value of the BaP analytical signal is characterized by a linear relationship on its concentration in the solution and is described by the equation $y = (4.011 \times 10^6)x$ ($R^2 = 0.9985$); the value of the analytical signal BeP is characterized also by the linear relationship on its concentration in the solution and is described by the equation $y = (1.495 \times 10^5)x$ ($R^2 = 0.9989$). The limit of quantitative determination (LQD) of every of substances being analyzed was found experimentally and is equal to the concentration of the matter creating analytical signal on the chromatogram (the peak of the matter), which by 10 times exceeds noise (signal/noise > 10). The LOQ of benzo(e)pyrene in melt water constitutes 1.0 and benzo(a)pyrene—0.01 μkg/dm^3. Comparing the values of tangents of inclination angles of graduation straight lines one may note that sensitivity of the method when analyzing BeP is much lower than the sensitivity established when analyzing BaP. In isomer benzopyrene mixtures with equal ratio of components the intensity of their signals differs 27 times.
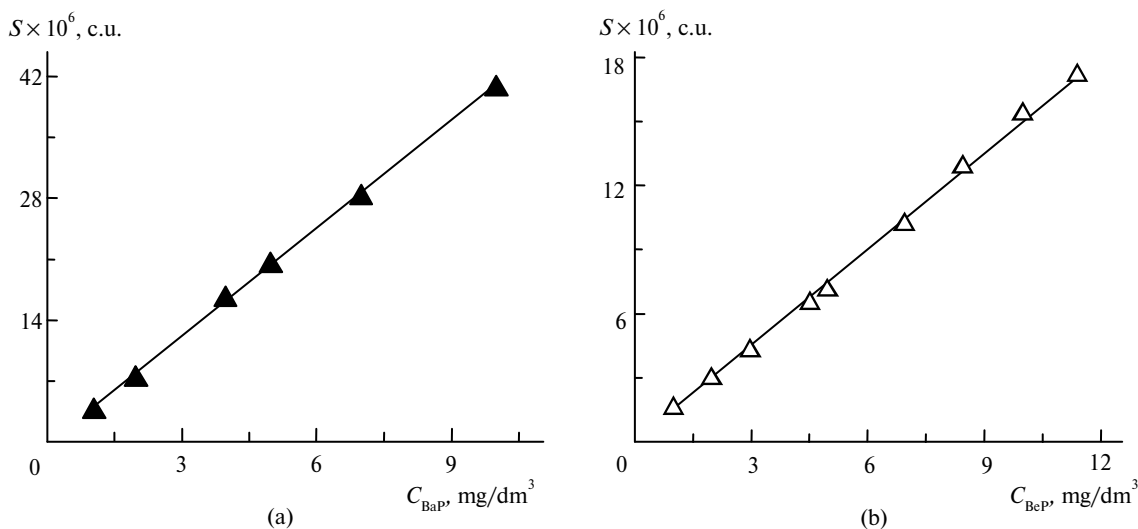

Fig. 1. Graduation relationship of the value of the peak area of the analytical substance on the chromatogram on the concentration of benzo(a)pyrene (a) and benzo(e)pyrene (b).

Thus, it was established that benzopyrene isomers may be measured by the HELC/FLD method. Analytical signals of isomers are not overlapped, but the BeP signal is 27 times weaker than BaP signal.

For measurement of PAH in atmospheric water snow samples were thermostated at 20°C for four hours. Sample preparation is based on BaP extraction, BeP and other PAH by mythylenechloride from melt water. Snow samples were taken near automobile roads and service stations, contained mechanical impurities and soot. When extracting these samples ultrasound effects were used and in this case organic layer acquired light-brown color stipulated by dissolution of soot. The color of the extract obtained from atmospheric water taken at a distance of 50 m from the automobile road corresponded to the color of a automobile pure extractant. The organic layer after separation of the water layer was evaporated changing the solvent. Obtained solutions in iso-propanol were subjected to chromatographic control.

Figure 2 shows a section of the chromatogram containing whole analytical substances and other PAH whose identification was made in a library formed by the authors earlier [13. 14]. From the said figure it is seen that the duration of maintaining BeP (peak *3*) in chromatographic analysis of its mixtures with BaP by benzo(b) fluoranten, chrisen and benzo(a)antracen constituted 39.5 min. Duration of holding BaP (peak *5*) in chromatographic analysis of PAH mixtures—45.7 min. In chromatographic analysis of isomer mixtures no overlapping of their analytical signals takes place as signals of four priority PAH. It means that the used technique is selective with respect to bensopyrene isomers. The results of measuring the content of PAH in atmospheric water obtained from snow taken near automobile roads and service stations and rural areas are given in Table 2.
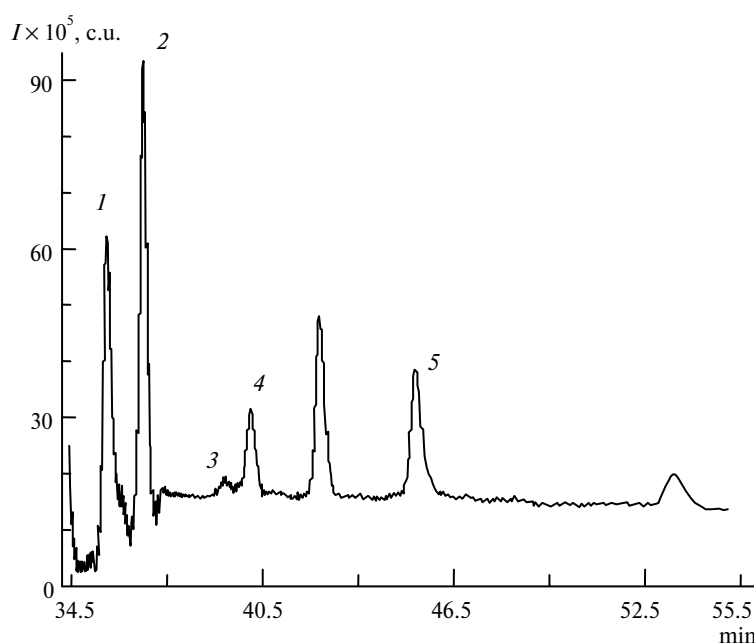

Fig. 2. Section of the chromatogram of the extract of atmospheric water obtained from the sample of snow sample taken along the automobile road. Identified substances: *1*—benzo(a)antracen, *2*—khrisen, *3*—benzo(e)pyrene, *4*—benzo(b)fluoranten, *5*—benzo(a)pyrene.

Table 2. Content of polycyclic aromatic hydrocarbons in the samples of atmospheric water sampled near automobile roads and service stations ($n = 3$, $P = 0.95$)

Substance, $\mu kg/dm^3$	PKO	PAH, mg/dm^3
Benzo(a)pyrene	0.01	0.50–1.07
Benzo(e)pyrene	1.0	1.0–2.5
Benzo(b)fluoranten	0.015	0.451–1.390
Khrisen	0.003	0.630–2.163
Benzo(a)antracen	0.005	0.649–3.468

Analyzing data given in this table one may come to a conclusion that snow sorbes polycyclic aromatic hydro-carbons. In its samples one could find isomers of benzopyrene in various mass ratios and also benzo(b)fuorant, chrisen and benzo(a)antracen. The most polluted sections were recorded near automobile roads and service stations. Samples of snow taken at a distance of 50 m from automobile roads did not contain PAH.

CONCLUSIONS

Thus, it was established that extraction from atmospheric water of benzopyrene isomers and other polycyclic aromatic hydrocarbons is accomplished by means of methylenechloride with subsequent concentration of the extract and replacement of the solvent by isopropanol. For measuring the content of benzopyrene isomers one should use the method of highly-effective liquid chromatography with fluorescent detection. The time of holding of benzopyrene isomers differs by 6.2 min, which makes it possible to determine individual substances, namely, benzo(e)pyrene and benzo(a)pyrene. Analytical signals of benzopyrene isomers are not overlapped by other polycyclic aromatic hydrocarbons identified in atmospheric water.

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