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Determination of PAHs in water based on multivariate analysis of fluorimetric data

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Polycyclic aromatic hydrocarbons (PAHs) are important environmental contaminants, which have been found in combustion products of synthetic fuels, cigarette smoke, etc. The carcinogenicity of some members of this group of compounds has been established. PAHs in environmental probe are invariably encountered as complex mixtures (including isomers, oxidized, alkylized substances etc.), the complete analysis of which is both time consuming and expensive because high resolution chromatographic and mass spectrometric techniques are needed. The possibilities of PAHs microliquid extraction from water and fluorimetric determination of the whole microextract volume have been investigated. Our approach based on chemometric analysis of excitation-emission matrix has been presented for the determination of PAHs and their mixtures in water samples. Various types of multivariate calibration (such as PCR, PLS etc.) were investigated. The influence of experimental condition on metrological characteristics of analysis was studied. Synchronous technique was also used for analysis of PAHs mixtures. The optimal experimental conditions for all consequence steps of determination of PAHs and their mixtures have been determined. The cost-effective, sensitive and fast technique for determination PAHs in water environmental samples has been developed and will be presented and discussed in detail.

Keywords: *multivariate calibration, PCR, PLS, PAH, fluorescence.*

Polycyclic aromatic hydrocarbons (PAHs) are well known group of environmental pollutants, which have been found in combustion products of synthetic fuels, cigarette smoke, etc. They are extremely hazardous, and this has led to the fact that sixteen of them were included by the Environmental Protection Agency on the list of priority pollutants. The carcinogenicity of some members of this group of compounds has been established. The drinking water regulation sets limiting concentration of 0.2 mg/L [1].

PAHs in environmental probe are invariably encountered as complex mixtures (including isomers, oxidized, alkylized substances etc.), the complete analysis of which is both time consuming and expensive because high resolution chromatographic and mass spectrometric techniques are needed. These analytical procedures are too costly, time-consuming, and labor intensive. Besides, efficient preconcentration of PAHs from the samples is indispensable before their determination because individual PAHs are presented in environment at the relative low concentration level.

The above-mentioned methods in use are highly sensitive and accurate which is unnecessary for the most part. It is known that only about 20% of water samples contain targeted pollutants, but in accordance with officially accepted methods all samples have to be analyzed according to time consuming procedures; the equipment used is very expensive. Cheapness, availability and efficiency of analysis are of great importance due to a large amount of analysis performed in environment pollution control.

General methodology of the environmental pollution control for targeted compounds seems to have to be changed. Reliable and cost effective PAHs pollution control has to include: fast screening of samples; estimation of pollutant contents instead of their accurate determination. Such control has to be based on the direct method of determination of targeted compounds which is characterized by low cost of equipment being used and simplifying of the analysis itself.

The luminescent spectrometry is the method, which is ideally suited for the determination of PAHs in environment control due to its sensitivity and simplicity.

However, the spectral behavior (overlapping of spectra of individual component) of complex mixtures of PAH in environmental samples does not allow us to realize the univariate calibration in the most real cases. Our approach based on chemometric analysis of excitation-emission matrix has been presented for the determination of PAH from samples containing their mixtures.

Experimental

Advantages of multivariate calibration were demonstrated on example of analysis of eight-

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component PAH mixture. Excitation-emission spectra (EES) of PAH hexane solutions were collected using spectrofluorimetr RF-530XPC (Shimadzu, Japan), 1cm quartz cell, slit width 3 nm and 20 nm. Scaning intervals were variated from 220 to 410 nm for excitation and from 220 to 700 nm for emission wavelength.

Results and discussion

We have used acenaphthene, anthracene, fluoranthene, benzo[a]pyrene, fluoranthene, fluorene, naphthalene, phenanthrene and pyrene as the model compounds. Each component has been presented by five concentration levels in calibration mixtures.

The spectral behavior of model PAHs in hexane has been investigated. It was demonstrated that the use EES allows us to obtain exhaustive information about mixtures under investigation. However, the absolute elimination of overlapping spectra of individual components and simultaneous determination of all model compounds based on univariate calibration are impossible.

The potentialities of methods of multivariate analysis have been investigated for simultaneous determination of PAHs in complex mixtures. Used methods are: classical least square method (CLS), principal component regression (PCR) and partial least square method (PLS2). Three-dimensional spectral data are transformed to two-dimensional matrix. In matrix notation, the CLS model is given by

$$X = C \cdot K ,$$

where X is the matrix of calibration spectra, C is the matrix of component concentrations, K is the matrix of coefficients [2]. The classical least-squares solution is

$$K = (C'C)^{-1} \cdot C'X ,$$

The PCR method combines the Principal Component Analysis (PCA) spectral decomposition with an Inverse Least Squares (ILS) regression method to create a quantitative model for complex samples. PCA decomposes an X matrix into two smaller matrices, one of scores (T) and the other of loadings (P) as follows:

$$X = T \cdot P'$$

Scores matrix T is converted onto concentration:

$$C = T \cdot R$$

where R is matrix of coefficients [3].

PLS, as PCR, is a quantitative spectral decomposition technique, but it uses the concentration information during the decomposition process:

$$X = T \cdot P'$$

$$C = T \cdot Q'$$

where Q is matrix of concentration loadings [4].

We have used cross-validation method leaving out one sample at a time for selection the optimal number of factors of PCR and PLS models. Cross-validation estimation also has been used for comparison potentialities of different models. The root mean square error is calculated as follows:

$$E = \sqrt{\sum_{i,j} \frac{(C_{ji} - \hat{C}_{ji})^2}{I}} ,$$

where C_{ji} – real concentration of component j in calibration mixture i, $_{ji}$ – predicted concentration of component j in calibration mixture i, I – total number of calibration mixtures [5].

It was found that root mean square errors calculated for cross-validation estimation for PCR and PLS2 non-significantly increased in instance of more poor spectral resolution (Tab. 1, Fig. 1). Such error for CLS calibration model demonstrates significant increase. Strong overlapping scattering peaks of solution and spectra of individual components of PAHs mixtures may explain this fact.

In order to reduce of root mean square errors of calibration model we have proposed to use only informative part of excitation-emission spectra of PAHs mixture (Fig. 2). These data are also transformed to two-dimensional matrix. Calibration models were developed for minimal data quantities, which included only maximum points of individual component spectra. The root mean square errors of calibration model are insignificantly changed, but it is required preliminary

Table 1. Root mean square errors (25 freedom degrees) of different calibration models.

Calibration model	High spectral resolution	Low spectral resolution
PCR	0.09	0.13
PLS2	0.09	0.14
CLS	0.09	0.29

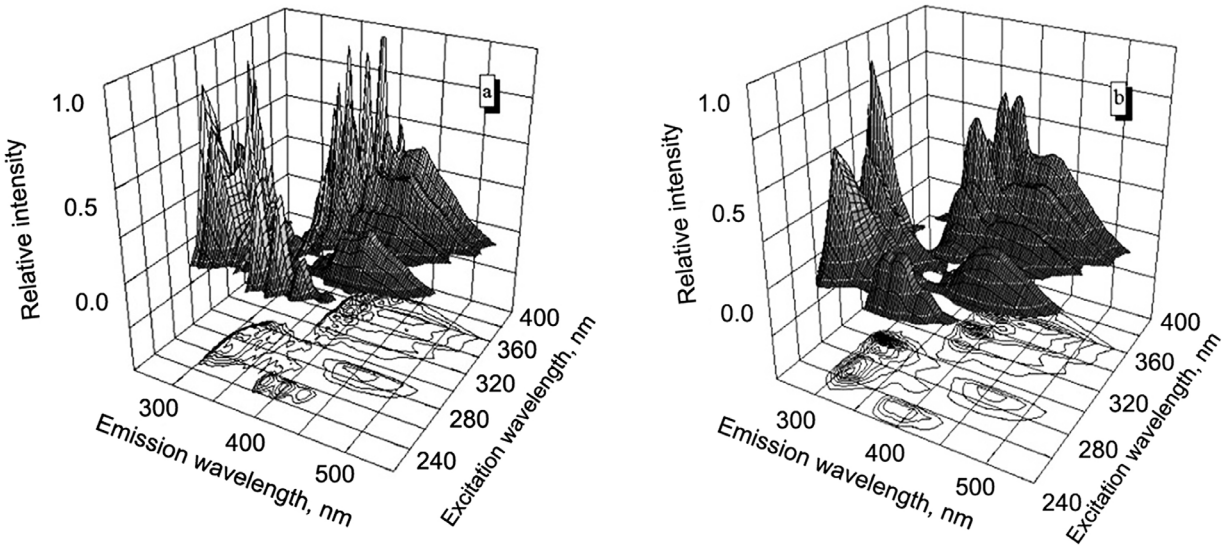

Fig. 1. Influence of spectral resolution on excitation-emission spectra of mixture including of eight PAHs: (a) emission monochromator slit 3 nm, (b) emission monochromator slit 20 nm.

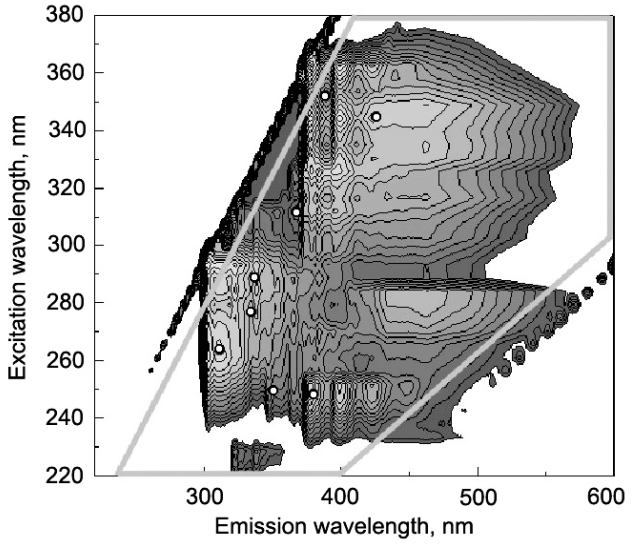

Fig. 2. Informative part of excitation-emission spectra of PAHs mixture (bold line) and maximum points of individual component spectra (marked with points).

investigation of excitation-emission spectra for this case. The root mean square errors of different calibration model are presented in Tab. 2.

Synchronous fluorimetry allows us to reduce the experimental data quantity and to simplify the spectral characteristics of investigated mixtures. We have used synchronous fluorimetric technique with constant value of difference between wavelengths emission and excitation. Preliminary analysis of emission-excitation spectra allows us to choose optimal value of such difference. In this case synchronous spectra contain information about all components, however overlapping of spectra of individual components still remains (Fig. 3).

Our investigation has demonstrated that relative standard deviation increased significantly (from 0.12 to 0.29) only for phenanthrene determination based on treatment of high-resolution synchronous spectra. In the case of treatment of low-resolution synchronous spectra the relative standard deviation of determination of all components was not changed in comparison with

Table 2. Root mean square errors (25 freedom degrees) of CLS, PCR and PLS2 calibration models, based on different raw data.

Calibration model	EES	Informative part of EES	Maximum points of individual component spectra
PCR	0.13	0.08	0.08
PLS2	0.14	0.08	0.08
CLS	0.29	0.08	0.08

Table 3. Root mean square errors (25 freedom degrees) of CLS, PCR and PLS2 calibration models for high resolution spectra.

Calibration model	Synchronous spectra	EES
PCR	0.15	0.09
PLS2	0.13	0.09
CLS	0.14	0.09

Table 4. Root mean square errors (25 freedom degrees) of CLS, PCR and PLS2 calibration models for low resolution spectra.

Calibration model	Synchronous spectra	EES
PCR	0.08	0.08
PLS2	0.08	0.08
CLS	0.08	0.08

Table 5. Relative standard deviation of determination PAHs in water samples (5 freedom degrees).

Compound	PCR	PLS2	CLS
Fluorine	0.09	0.09	0.09
Anthracene	0.08	0.08	0.09
Fluoranthene	0.07	0.07	0.07
Acenafthene	0.07	0.07	0.08
Pyrene	0.04	0.04	0.05
Phenanthrene	0.06	0.06	0.06
Naphthalene	0.05	0.06	0.06
Benzo[a]pyrene	0.15	0.15	0.15
Total root mean square error	0.08	0.09	0.09

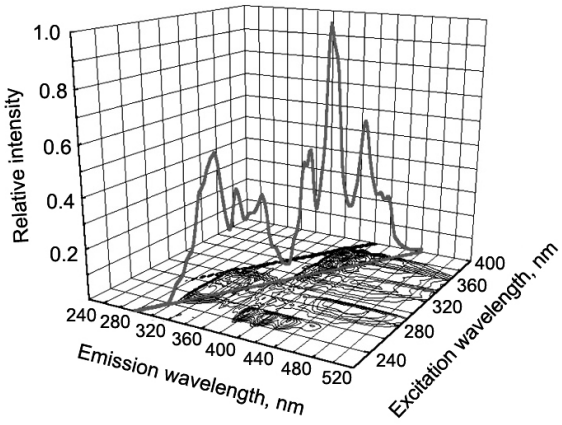

Fig. 3. Synchronous fluorescence spectra of PAHs mixture with constant value of difference between emission and excitation wavelengths.

treatment of excitation-emission spectra. It should be noted that such spectral data are more informative for multivariate calibration (Tab. 3, 4).

The approach developed by us was tested for simultaneous determination of components of complex PAHs mixtures in real water samples. Targeted compounds were extracted into hexane (water/organic phase ratio was 1:1). Ultrasound treatment of contacting phases was used for accelerating extracting procedure. It was found that the root mean square errors of investigated calibration models differed insignificantly from cross-validation error. This fact is an evidence of adequacy of developed calibration models (Tab. 5).

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