Contribution to the Fluorometric Method of Analysis for Atmospheric Benzo(a)pyrene

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Summary. The potentiality of the method for the determination of benzo(a)pyrene (BaP) in airborne particulates, based on the fluorescence measurements in H\textsubscript{2}SO\textsubscript{4}, was investigated. Stability of the BaP fluorescence in H\textsubscript{2}SO\textsubscript{4}, isolation of the BaP fraction by thin-layer chromatography and the effect of quenchers on the fluorescence intensity of BaP were studied and the results are discussed. The relative standard deviation of the method was determined to be 2.9 and 5.9\% for BaP levels of 265 and 68 ng, respectively.

Best. von Benzo(a)pyren in Luft; Fluorimetrie; DC-Trennung, H\textsubscript{2}SO\textsubscript{4}-Lösung.

Introduction

During the last two decades a great number of methods for the determination of atmospheric benzo(a)pyrene (BaP) has been developed. They differ in their precision, accuracy, selectivity, sensitivity, and requirements for the analysis time and instrumental equipment [4,9]. Methods based on fluorescence measurements are the most sensitive ones. Selectivity of these methods can be enhanced when using concentrated sulphuric acid as a solvent since the fluorescence of BaP in this acid is much more specific than that in organic solvents. For the first time the utilization of sulphuric acid was described by Sawicki et al. [6] for the determination of BaP in complex mixtures. Since that time the method has been studied thoroughly and some changes and improvements have been made [2, 3, 5]. We have recently investigated the potentiality of this method before introducing it to our laboratory. This paper describes the results of our research and is supposed to assist in the development of a very simple, sensitive and selective method for analyzing the atmospheric BaP.

Experimental

1. Extraction of Samples

A sublimation method was successfully employed for the extraction of BaP. The glass fibre filters with airborne particulates were sublimed in vacuum at 315°C for 45 min [1].

2. Thin-Layer Chromatography of Sample Extracts

Extracts of samples were concentrated by reducing their volume to about 0.2 ml in vacuum and applied as a streak on a silica gel coated aluminium foil SILUFOL (Kalvailer Votice, ČSSR). The foil sheet was developed with the mixture n-heptane/benzene (80 : 20). BaP as a standard was run alongside the sample. After development the area of the foil, where BaP occurred, was marked under UV light, cut off, further cut into small pieces, transferred into a test-tube and eluted three times always with 3 ml of cyclohexane + 1 drop of N,N-dimethylformamide (DMF).

3. Fluorescence Measurements

The fluorometric determination of BaP in the cyclohexane eluates was carried out on the one hand by means of direct reading from a standard curve and on the other hand with the help of the standard addition method.
a) Direct Reading from a Standard Curve (SCM). The cyclohexane eluate (9 ml of cyclohexane + 1 drop of DMF) was filtered into a 50-ml separatory funnel and 3 ml of conc. H₂SO₄ were added. After 1 min of shaking the lower layer was transferred into a glass cuvette and the fluorescence intensity of this solution was measured in the SPEKOL Zeiss spectrophotometer equipped with a fluorescence attachment and with a three-stage photo-multiplier. The activating wavelength was set at 522 nm and the emission wavelength was selected by means of an interference filter peaking at 540 nm with the half-width of 4.5 nm. The concentration of BaP was obtained from the standard curve which was a straight line up to a concentration of 1000 ng/3 ml. The coordinates of the curve were the relative intensity of fluorescence and the total BaP concentration in 3 ml of H₂SO₄. The lowest detectable concentration of BaP was of 5 ng/3 ml of H₂SO₄.

b) Standard Addition Method (SAM). The cyclohexane eluate was filtered into a 10-ml volumetric flask and made up to the mark. From there, three 3-ml portions were transferred into three 50-ml separatory funnels and to two out of them different but known amounts of standard BaP solution were added. Further, 3 ml of conc. H₂SO₄ were added to each separatory funnel and after shaking the fluorescence intensities were measured. From these results the concentration of BaP was obtained graphically.

Results and Discussion

1. Examination of the stability of BaP fluorescence in H₂SO₄ showed that the intensity is stable for at least 15 min in the concentration range of 90 to 4000 ng/3 ml of BaP, without using nitrogen-purged sulphuric acid solvent. In lower concentrations the fluorescence became satisfactorily stable 10 min after adding H₂SO₄.

2. Isolation of BaP Fraction by Thin-Layer Chromatography. We have found that the results obtained by direct fluorescence measurement in H₂SO₄ without TLC separation encounter an error varying between −17% and +43%.

To test the effect of other chemical substances present in airborne particles we separated 2 ml of sample extract by a previously described TLC method and divided the spots obtained into five sections marked by numbers 1 to 5. The section No.3 contained BaP fraction, No.2 contained compounds lying just above the BaP and No.4 compounds lying just under the BaP. The sections Nos.1 and 5 contained compounds occurring in the rest of the chromatogram upward and downward from the BaP, respectively. All sections were cut off, eluted, extracted into H₂SO₄ and the relative fluorescence intensity was measured (Fig.1). When summing the relative fluorescence intensities of all sections we obtained the total value of 137, including the value of 58 for BaP. These results indicate how much compounds other than BaP can contribute to the total fluorescence of the sample extract even at the spectrophotofluorometer setting for the BaP analysis (Ex 522 nm, Em 540 nm). We could expect that without chromatographic separation we would obtain more than twofold the concentration of BaP. This would be true, if there were not the simultaneous quenching effect of those substances. When another 2 ml portion of the same sample extract was analyzed for BaP without TLC separation a relative fluorescence intensity of only 51 was obtained. In this case the difference represents a negative error of 14%.

For these reasons the TLC separation of the BaP fraction is inevitable when accurate results are required. The employed TLC procedure does not completely isolate BaP from other polynuclear aromatics (PNA's) but according to the data which have been so far reported [6–8] and based on our own experience, none of the other PNA's with an Rf similar to that of BaP fluoresces in H₂SO₄ under the conditions used for the BaP analysis.

TLC recovery studies with pure BaP on silicagel-coated aluminium foils indicated an value of 89% for triple extraction with 3 ml of cyclohexane + 1 drop of DMF. The efficiency of this elution mixture is very high and the eluate is readily extractable with H₂SO₄. To eliminate the error resulting from losses of BaP during TLC, standards of pure BaP were run through the whole chromatographic procedure and the measured relative fluorescence intensities were used to plot the calibration curve.

3. Effect of Quenchers on Fluorescence Intensity of BaP. The non-separated PNA's from BaP have no