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ANALYSIS OF MUTAGENIC NITROARENES IN CARBON BLACK BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A method was developed for the analysis of mutagenic nitroarenes in carbon black. The procedure included extraction, pre-separation column chromatography on silica gel, and determination by high-performance liquid chromatography (HPLC) using a diode-array detector. Different solvents, such as dichloromethane, benzene, toluene, and chlorobenzene were used for the extraction of carbon black. It was found that chlorobenzene yielded the highest amount of extracted material. The pre-separation, the optimization of the HPLC separation, the selection of detection wavelengths, and the calibration curve are discussed. 3-Nitro-9-fluorenone was detected in a treated carbon black, which is used for making carbon ink. The identification of peaks in the sample chromatograms was confirmed by comparing the retention time and the UV spectra with those of standards.

INTRODUCTION

The isolation and identification of nitroarenes has been the subject of many recent research papers, since it was demonstrated that these compounds show a direct activity in the Ames test[1], a rapid test designed to identify potential mutagenic or carcinogenic chemicals. Xu *et al.* have isolated and identified more than 50 nitroarenes in the extracts of diesel-exhaust particulates[2] and found 1-nitropyrene and other nitroarenes in airborne particulate matters[3]. It was reported by Löfroth *et al.*[4] that the extracts of selected xerographic toners produced positive responses in the Ames Salmonella mutagenicity assay. This activity was traced to various nitropyrenes in the carbon black used as the toner colorant. Jin *et al.*[5] found mutagenic activity in an organic extract of a treated carbon black, which is the main component of carbon ink. However, the determination of nitroarenes in the environmental samples as well as in carbon black samples has proved to be a difficult task, because of their low concentration and because the samples contain a vast number of potentially interfering compounds. Methods are required that are not only extremely sensitive but also provide high selectivity. Capillary-column gas chromatography (GC)[6], gas chromatography–mass spectrometry (GC–MS)[2,7], thin-layer chromatography (TLC)[8], MS–MS[9], and high-performance liquid chromatography (HPLC)[10,11] have been applied to the analysis of nitroarenes in complex samples. Decomposition of

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nitroarenes in GC or GC–MS systems has made positive identification and quantification difficult at the low levels present in the complex samples[12].

In the present paper, a simple analytical procedure is described, which includes extraction of the sample with organic solvents, pre-separation by column chromatography on silica gel, and the separation and determination by reversed-phase HPLC with a UV detector. The pre-treatment of samples, the separation by HPLC, and the identification of chromatographic peaks are discussed.

EXPERIMENTAL

Chemicals and standards

All solvents were of analytical grade and distilled in glass before use. Methanol, dichloromethane, benzene, toluene, and monochlorobenzene were obtained from Beijing Chemical Works (Beijing, China). Hexane was obtained from First Tianjing Reagent Works (Tianjing, China). Sep-Pak silica cartridges were obtained from Waters Associates (Milford, MA, U.S.A.). Redistilled water was used.

2-Nitrobiphenyl (2-NB), 4-nitrobiphenyl (4-NB), 2-nitrofluorene (2-NF), 3-nitro-9-fluorenone (3-NFO), 2,7-dinitrofluorene (2,7-DNF), 2,7-dinitro-9-fluorenone (2,7-DNFO), 3-nitrofluoranthene (3-NFA), 9-nitroanthracene (9-NA), and 1-nitropyrene (1-NP) were obtained from Aldrich (Milwaukee, WI, U.S.A.). All reference standards were used without purification.

HPLC apparatus

The chromatographic apparatus consisted of a Model 1090 solvent delivery system, a Model 79846A autoinjector, a Model DAD diode-array detector, a Model HP-85B personal computer, and a Model 3390A integrator, all from Hewlett-Packard, Palo Alto, CA, U.S.A. A DuPont Zorbax-ODS (25 cm × 4.6 mm) reversed-phase column was used for all separations. Chromatography was carried out with mixtures of water and methanol as the mobile phase.

Samples and preseparation

Both original (C_1) and treated carbon black (C_2) samples were obtained from Tu Yuemin of Beijing Ink Works, Beijing, China. The original carbon black was manufactured by Nan Ping Carbon Black Works (Nan Ping, Fujian, China). The treated carbon black is the product of the reaction of the original carbon black with diluted nitric acid. The pre-treatment of samples has been described elsewhere[5]. In short, the procedure includes extraction with organic solvents, concentration, and pre-separation with a Sep-Pak silica gel cartridge. The fraction of medium polarity, which contained nitroarenes, was separated and analyzed by HPLC.

High-performance liquid chromatography

The diode-array detector was used for optimization of chromatographic signals in seven wavelength ranges, and for confirmation of the identity of sample peaks by comparing their retention times and the UV spectra with those of standards. The external standard method was applied to the quantitative analysis with the integrator.

RESULTS AND DISCUSSION

Extraction and preseparation

Soxhlet extraction with organic solvents was used to isolate the organic matter from carbon black samples in this work, because it has been shown that some compounds are degraded during vacuum sublimation[13]. Of the four solvents initially investigated for extraction, the highest overall yield of organic matter was obtained with chlorobenzene, as shown in Table I.

TABLE I

THE OVERALL YIELD (%) OF SOXHLET EXTRACTION WITH DIFFERENT SOLVENTS

Sample	CH_2Cl_2	C_6H_6	$C_6H_5CH_3$	C_6H_5Cl
C_1	0.117	0.210	0.336	0.550
C_2	0.416	0.406	0.802	0.861

Higher extraction yields were obtained from treated carbon black sample C_2 than from the original sample C_1 with all four solvents. It seems that treated carbon black contains more soluble substances.

The extracts of carbon black may contain a number of impurities related to polynuclear aromatic hydrocarbons[14]. In order to isolate the medium-polar nitroarene fraction of the extract, a silica gel cartridge was applied for pre-separation, as described in the previous paper[3]. Briefly, after the extract was deposited on the top of a silica gel cartridge, the cartridge was eluted successively with hexane, dichloromethane, and methanol. The fraction eluted by dichloromethane was concentrated, dried, diluted with methanol, and analyzed by HPLC.

High-Performance liquid chromatography of nitroarenes

The reversed-phase ODS column was used to separate the nitroarenes. The separation conditions were optimized by changing the strength of the mobile phase at the suitable detection wavelength. The capacity factors, k', of nitroarene standards as a function of the methanol concentration are shown in Fig. 1. It is seen that the standard mixture can be well separated by isocratic elution with 75% aq. methanol.

Fig. 2 shows the separation of nine nitroarene standards under the HPLC conditions described above at a detection wavelength of 260 nm, which was chosen by comparing the chromatographic signals at seven wavelengths (Fig. 3). The detection responses of nitroarenes at 260 nm were linear up to 30 ng. The specific responses of nitroarenes decreased in the following order: 1-NP, 2,7-DNF, 9-NA, 3-NFO, 3-NFA, 2,7-DNFO, 4-NB, 2-NB, and 2-NF.

Nitroarenes in carbon black

Organic elemental analysis indicated that only the treated carbon black sample, C_2, contained nitrogen. The chromatogram of the extract of sample C_2, shown in Fig. 4, contained peaks, labeled a–d, which had retention times close to those of reference 2,7-DNFO, 3-NFO, 4-NB and 9-NA, respectively. Additional information

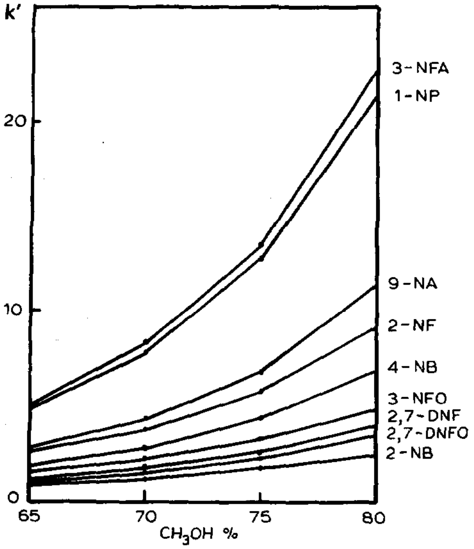
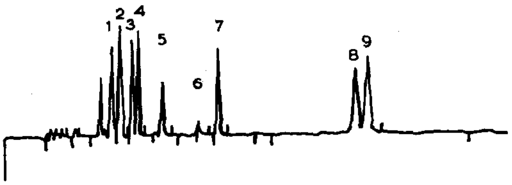


Fig. 1. The effect of the concentration of methanol in the mobile phase on the capacity factor, k'.

concerning the identities of peaks a–d was obtained by comparing the UV spectra of these peaks with those of the suspected reference nitroarenes. The only candidate nitroarene now confirmed is 3-NFO, which produces a UV spectrum matching that of peak b within the range of experimental error (see Fig. 5). The spectra of the other candidate nitroarenes were different from those of the unknown peaks.

For the determination of the concentration of 3-NFO in the extract of sample C_2, the recovery of 3-NFO was determined by spiking a carbon black extract with the standard, and then following the procedure described in Experimental. The con-



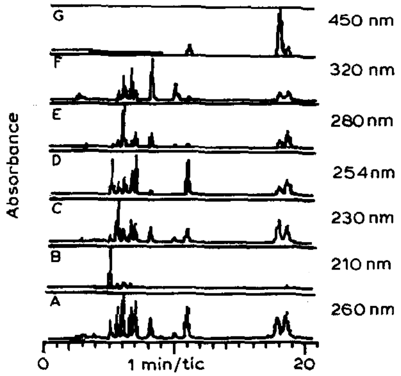


Fig. 2. Chromatogram of nine reference nitroarenes. HPLC conditions: Zorbax ODS (25 cm × 4.6 mm), 75% aq. methanol, detection at 260 nm. Peaks: 1 = 2-NB, 2 = 2,7-DNFO, 3 = 2,7-DNF, 4 = 3-NFO, 5 = 4-NB, 6 = 2-NF, 7 = 9-NA, 8 = 1-NP, and 9 = 3-NFA.

Fig. 3. Comparison of chromatographic signals at seven detection wavelengths.

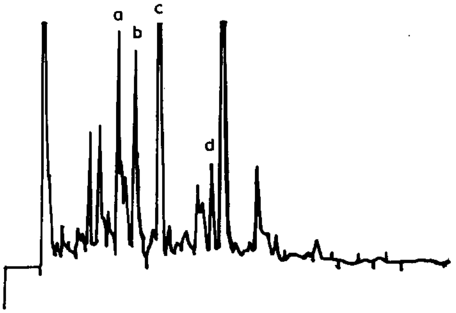


Fig. 4. Chromatogram of a 2-μg portion of extract of carbon black sample C_2. Chromatographic conditions as in Fig. 2.

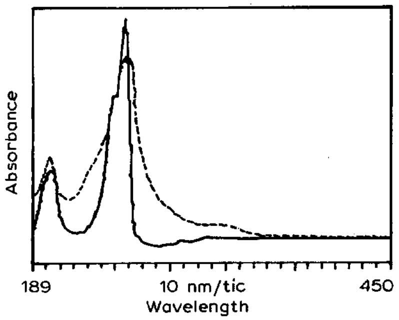


Fig. 5. The UV spectra of peak b (Fig. 4) (———) and of reference 3-NFO (— — —), which has the same retention time as that of peak b.

centration of 3-NFO in the extract of treated carbon black sample C_2 after correction for a recovery of 90% was 0.25 ± 0.02%, which is equal to 21.5 ± 0.5 ppm.

The mutagenic activity of 3-NFO has been evaluated in the previous paper[15]. More attention should be paid to the health effects on people who are exposed to treated carbon black.

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