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Quantitative determination of 3,4-benzopyrene in the air near gas-works retorts

In two earlier papers extracts of industrial air particulates were purified by two-dimensional thin-layer chromatography prior to examination by gas chromatography[1] or UV-absorption spectroscopy[2]. The thin-layer system described by KÖHLER *et al.*[3] was used with some minor modifications[2]. In the earlier work[1], a quantitative analysis of the 3,4-benzopyrene fraction extracted from the thin layer was attempted with the aid of an electron capture detector attached to the gas chromatograph. However, it was found that the recovery was not reproducible and varied between 60 and 95%. It was found later that losses occurred during both the purification procedure and the gas chromatographic analysis. Fluctuations were also found in the sensitivity of the electron capture detector, very likely due to imperfect purification.

In this study smaller thin-layer plates (10 × 10 cm) have been used, and this is found to reduce the losses in the purification procedure. In order to improve the reproducibility of the detector response, the heating system of the detector of the commercial gas chromatograph was modified so that a constant temperature (mostly 185°) could be maintained despite a draught in the laboratory. Glass liners inserted in the ends of the glass columns minimized decomposition due to contact with metal surfaces. Since the benzopyrene fractions extracted from the thin layers were also analyzed by absorption spectroscopy errors occurring at some steps of the analysis were easily found.

Experimental

Reagents. The following reagents were used: benzene p.a. (Merck); toluene p.a. (Merck); *n*-hexane puriss min. 99% (Kebo); methanol p.a. (Merck); anhydrous ether A.R. (Mallinckrodt); Silica Gel H according to Stahl (Merck); Cellulose Powder MN 300 Ac., acetyl content approx. 40% (Macherey, Nagel & Co.); and ethyl alcohol, 95%.

The benzene was redistilled before use; the first and last 10% of the distillate was discarded. All reagents used were frequently checked by blanks.

Apparatus. The following apparatus was used: Desaga–Stahl kit for the preparation of thin layers on 20 × 20 cm glass plates; Varian-Aerograph Model 204 gas chromatograph with 250 mCi tritium source electron capture detectors; Leeds and Northrup Speedomax H 1 mV recorders; Beckman DBG UV–visual spectrophotometer equipped with recorder S.03507H, Spectrosil semimicro cells of 1 cm path length and variable beam attenuators.

Thin-layer chromatography. An air particulate extract, as used in previous qualitative work[2], was diluted to give a 3,4-benzopyrene concentration of about 20 ng/μl.

Sufficient slurry to cover five 20 × 20 cm glass plates with a thin layer 0.25 mm thick was prepared from 25 g silica gel, 12 g cellulose acetate and 65 g 95% alcohol by stirring mechanically at about 1000 r.p.m. for 5 min. After drying in air for 10 min, the plates were heated in an oven at 120° for 30 min. The 20 × 20 cm adsorbent layers were then divided into four 10 × 10 cm sections by scraping with a stylus. Four plates with sixteen 10 × 10 cm layer sections were now treated as follows.

1.0 μl of the diluted benzene extract was applied to the outer corner of 15 of the layer sections, 15 mm from each side of the plate, to give a spot diameter of not more than 3 mm. Standard additions of 50 ng of 3,4-benzopyrene were then made to 12 of the 15 starting points. The 16th layer section was used as a blank. The four plates were now placed in a tank to run eight thin-layer sections in the first direction. After turning the plates, the remaining eight sections were run in the same tank. The second direction runs were performed in the corresponding way. The mobile phases were *n*-hexane–toluene (9:1) first direction, and ether–methanol–water (4:4:1) second direction. After completion of the runs, the thin layers were dried in air. On examination under a 254-nm UV-lamp, the fluorescent B*a*P-spots could be outlined with a stylus.

The adsorbent in the outlined areas was collected in suction tubes[2] from which the benzopyrene fractions were extracted by five successive 0.2-ml quantities of benzene[2]. The solvent was then evaporated in a stream of nitrogen and the fraction dissolved in 200 μl of benzene.

Using the same procedure three more series of thin layers were run with double the amount of particulate extract and a standard addition of 100, 200 and 400 ng of 3,4-benzopyrene, respectively. In the 400 ng series the thickness of the thin layers was increased to 0.40 mm and the fraction was dissolved in 300 μl of benzene.

Spectrophotometry. After the addition of the solvent, the test tube was gently shaken and the solution immediately transferred to the sample cell with a pipette. The scanning range was 430–350 nm and pure benzene was used as reference since no background absorption appeared when scanning the blank sample. The absorption peaks were enlarged up to five times with a scale expander. After the scan, two 5 to 7 μl portions were removed from the sample cell with two microsyringes for examination by gas chromatography. After injection, a second scan was made and then another two portions were removed.

Gas chromatography. The columns were silanized pyrex glass tubes, 1 m length, with an O.D. of 3 mm and an I.D. of 1.8 mm, and the coil has a diameter of 100 mm. The column packing was silanized Gas-Chrom P (100–120 mesh) coated with 6% QF-1. The columns were conditioned for 48 h at 225° with an inlet nitrogen pressure of 0.5 atm.

For the GLC separation of the 3,4-benzopyrene in the TLC fraction, the column temperature was set to 195° and inlet nitrogen pressure to 2.0 atm, giving a flow of about 30 ml/min. Injection and detector temperatures were 220° and 185°, respectively.

In order to prevent thermal decomposition, the steel capillaries normally joining the columns to the detectors were disconnected and the columns were connected directly to the detector sockets. For the same reason, the front ends of the columns and the injector glass liners were joined with teflon tubes.

To reduce the response variation errors, an injection of a standard was made after four sample injections. The two channels of the gas chromatograph were in this respect considered as two individual chromatographs, marked as ECD A and ECD B, respectively, *cf.* Tables I and II.

Results and discussion

When silica gel was used instead of aluminium oxide in order to reduce tailing in the first direction[1] the retention value of 2,3-(*o*-phenylene)pyrene, present in the particulate extract, coincided with that of 3,4-benzopyrene[2]. Consequently, the 3,4-

benzopyrene fraction, *i.e.* the TLC containing all the visible 3,4-benzopyrene contained also small quantities of both 3,4-benzofluoranthene and 2,3-(*o*-phenylene)-pyrene. These two contaminants are easily separated from 3,4-benzopyrene in the GLC column but must be corrected for in spectrophotometry. This is done by measuring the absorbancy of the 3,4-benzopyrene fraction at three wavelengths. If BP, BF and PP denote 3,4-benzopyrene, 3,4-benzofluoranthene and 2,3-(*o*-phenylene)pyrene and A, C and ε denote absorbance, concentration and absorptivity, the following equations are valid, according to Beer's law, for mixtures of the three polyaromatic hydrocarbons.

$$A_{\lambda n} = \varepsilon_{\lambda n}^{BP} C^{BP} + \varepsilon_{\lambda n}^{BF} C^{BF} + \varepsilon_{\lambda n}^{PP} C^{PP} \qquad n = 1, 2, 3.$$

Elimination of C^{BF} and C^{PP} gives

$$C^{BP} = k_1 A_{\lambda 1} + k_2 A_{\lambda 2} + k_3 A_{\lambda 3}$$

TABLE I

GAS CHROMATOGRAPHIC ANALYSIS OF BaP; CHANNEL A

Amounts of BaP in the TLC fractions and deviations from mean values found by analysis by GC, ECD channel A.

TLC fraction	*Standard addition*							
	400 ng		*200 ng*		*100 ng*		*50 ng*	
	x_i (*BaP found*)	$x_i - \bar{x}$	x_i (*BaP found*)	$x_i - \bar{x}$	x_i (*BaP found*)	$x_i - \bar{x}$	x_i (*BaP found*)	$x_i - \bar{x}$
1	423	19	213	4	113	−1	60	1
	406	2	214	5	109	−5	61	2
2	410	6	208	−1	109	−5	64	5
	410	6	211	2	114	0	64	5
3	411	7	212	3	113	−1	58	−1
	402	−2	209	0	113	−1	59	0
4	403	−1	195	−14	113	−1	59	0
	375	−29	202	−7	113	−1	64	5
5	400	−4	217	8	110	−4	60	1
	409	5	216	7	113	−1	60	1
6	405	1	227	18	111	−3	56	−3
	414	10	214	5	107	−7	60	1
7	395	−9	212	3	108	−6	60	1
	406	2	202	−7	106	−8	55	−4
8	392	−12	218	9	113	−1	57	−2
	384	−20	216	7	113	−1	54	−5
9	379	−25	205	−4	130	26	58	−1
	410	6	205	−4	123	9	55	−4
10	399	−5	214	5	125	11	56	−3
	409	5	210	1	124	10	58	−1
11	401	−3	208	−1	—	—	56	−3
	405	1	207	−2	—	—	54	−5
12	418	14	192	−17	—	—	63	4
	428	24	197	−12	—	—	65	6
01	31	—	26	—	33	—	16	—
	31	—	28	—	29	—	16	—
02	29	—	28	—	31	—	15	—
	31	—	27	—	28	—	15	—
03	29	—	28	—	29	—	14	—
	26	—	28	—	27	—	14	—

where k_1, k_2 and k_3 can be calculated from ε-values. The absorptivities for 3,4-benzopyrene were determined by scanning six standard solutions, varying from 200 to 1200 ng per 200 μl. The plot of absorbance *versus* concentration showed that Beer's law was obeyed. Absorptivities for 3,4-benzofluoranthene and 2,3-(*o*-phenylene)pyrene were taken from the spectrum of each.

Thus, it was found that the amount of 3,4-benzopyrene in the TLC fraction, when dissolved in 200 μl of benzene, was given by the expression

$$1940\ (A_{\lambda 1} + 1.09\ A_{\lambda 2} - 2.21\ A_{\lambda 3})\ \text{ng}$$

The wavelengths chosen were two maxima and one minimum on the 3,4-benzopyrene absorption curve at 390, 370 and 380 nm, respectively.

For GLC analysis, the amounts of standard and sample injected were such that they would not give more than 5 ng of 3,4-benzopyrene, that is about half the maximum

TABLE II

GAS CHROMATOGRAPHIC ANALYSIS OF BaP; CHANNEL B

Amounts of BaP in the TLC fractions and deviations from mean values found by analysis by GC, ECD channel B.

TLC fraction	*Standard addition*							
	400 ng		*200 ng*		*100 ng*		*50 ng*	
	x_i (*BaP found*)	$x_i - \bar{x}$	x_i (*BaP found*)	$x_i - \bar{x}$	x_i (*BaP found*)	$x_i - \bar{x}$	x_i (*BaP found*)	$x_i - \bar{x}$
1	406	24	217	16	121	1	59	−2
	422	40	189	−12	120	0	60	−1
2	390	8	199	−2	122	2	63	2
	343	−39	209	8	117	−3	64	3
3	400	18	212	11	118	−2	64	3
	386	4	202	1	106	−14	63	2
4	379	−3	218	17	119	−1	62	1
	378	−4	219	18	122	2	63	2
5	384	2	208	7	118	−2	63	2
	325	−57	203	2	117	−3	63	2
6	398	16	196	−5	125	5	63	2
	376	−6	197	−4	126	6	62	1
7	389	7	190	−11	117	−3	60	−1
	368	−14	178	−23	107	−13	56	−5
8	422	40	206	5	116	−4	58	−3
	401	19	200	−1	119	−1	59	−2
9	386	4	204	3	126	6	62	1
	346	−36	186	−15	130	10	60	−1
10	383	1	204	3	129	9	61	0
	359	−23	194	−7	127	7	60	−1
11	386	4	197	−4	—	—	59	−2
	386	4	200	−1	—	—	59	−2
12	374	−8	204	3	—	—	64	3
	389	7	188	−13	—	—	62	1
01	34	—	31	—	34	—	19	—
	36	—	31	—	32	—	18	—
02	33	—	29	—	32	—	16	—
	33	—	26	—	32	—	18	—
03	30	—	34	—	31	—	19	—
	31	—	31	—	28	—	17	—

TABLE III

SPECTROPHOTOMETRIC DETERMINATION OF BaP

Values for $A = A\lambda_1 + 1.09\,A\lambda_2 - 2.21\,A\lambda_3$ for the TLC fractions and deviations from the mean values found by absorption spectroscopy.

TLC fraction	*Standard addition*							
	400 ng		*200 ng*		*100 ng*		*50 ng*	
	$A_i \cdot 10^4$	$(A_i - \bar{A}) \cdot 10^4$	$A_i \cdot 10^4$	$(A_i - \bar{A}) \cdot 10^4$	$A_i \cdot 10^4$	$(A_i - \bar{A}) \cdot 10^4$	$A_i \cdot 10^4$	$(A_i - \bar{A}) \cdot 10^4$
1	2073	21	1136	35	643	21	324	0
	2073	21	1136	35	643	21	302	−22
2	2068	16	1115	14	615	−7	328	4
	2015	−37	1119	18	626	4	327	3
3	2060	8	1098	−3	615	−7	334	10
	2093	41	1119	18	609	−13	333	9
4	2045	−7	1101	0	611	−11	324	0
	2045	−7	1134	33	626	4	317	−7
5	2039	−13	1125	24	615	−7	324	0
	2039	−13	1127	26	615	−7	322	−2
6	2039	−13	1097	−4	600	−22	328	4
	2069	17	1133	32	611	−11	328	4
7	2016	−36	1046	−55	626	4	322	−2
	2069	17	1068	−33	640	18	337	13
8	2023	−29	1112	11	600	−22	314	−10
	2051	−1	1109	8	601	−21	309	−15
9	2078	26	1075	−26	632	10	329	5
	2044	−8	1075	−26	644	22	308	−16
10	2068	16	1064	−37	629	7	323	−1
	2068	16	1109	8	622	0	344	20
11	2010	−42	1080	−21	—	—	317	−7
	2062	10	1080	−21	—	—	328	4
12	2069	17	1093	−8	—	—	335	11
	2038	−14	1068	−33	—	—	323	−1
01	167	—	150	—	162	—	92	—
	172	—	136	—	170	—	81	—
02	157	—	148	—	158	—	86	—
	160	—	154	—	158	—	83	—
03	158	—	176	—	154	—	91	—
	154	—	173	—	163	—	91	—

amount for the linear response on the ECD. When two equal standard injections showed different peak heights, the response for the sample injections inbetween were calculated by interpolation according to the number of injections after the first standard.

The amount of 3,4-benzopyrene in the TLC fractions analyzed determined with the two GLC channels and deviations from mean values are listed in Tables I and II. Table III shows the values found and deviations from mean values for $A = A_{\lambda_1} + 1.09\,A_{\lambda_2} - 2.21\,A_{\lambda_3}$. By subtracting the mean values obtained by the analysis of fractions 01, 02 and 03 from the corresponding total values, the mean recovery values were calculated. They are listed in Table IV together with their standard deviations. Thus Table IV illustrates the results of the present investigation.

It is obvious that, in determinations of 3,4-benzopyrene with the combination of TLC–GLC described, the GLC system is the major source of statistical errors. On the thin layer, some of the 3,4-benzopyrene is only eluted by the mobile phase of the

TABLE IV

COMPARISON OF GAS CHROMATOGRAPHIC AND SPECTROMETRIC DETERMINATION OF BaP

Mean total amounts and mean absorbances of BaP (and standard deviations) in the TLC fractions found by gas chromatography and absorption spectroscopy Standard additions of BaP and mean recoveries are also listed

Standard addition	*ECD A*			*ECD B*			*Absorption spectroscopy*		
ng	$\bar{x}$ *(BaP found)*	$\left(\frac{(x_i-\bar{x})^2}{n(n-1)}\right)^{\frac{1}{2}}$	*Recovery (%)*	$\bar{x}$ *(BaP found)*	$\left(\frac{(x_i-\bar{x})^2}{n(n-1)}\right)^{\frac{1}{2}}$	*Recovery (%)*	$\bar{A}$ *(absorbance found)*	$\left(\frac{(A_i-\bar{A})^2}{n(n-1)}\right)^{\frac{1}{2}}$	*Recovery (%)*
	(ng)			*(ng)*					
400	404	3	94 ± 1	382	5	87 ± 2	0 2052	0.0005	92 ± 1
200	209	2	91 ± 1	201	3	85 ± 2	0 1101	0 0006	92 ± 1
100	114	2	85 ± 2	120	2	89 ± 2	0 0621	0.0004	90 ± 1
50	59	1	88 ± 2	61	0.5	87 ± 1	0 0324	0.0003	92 ± 1

second direction. This effect may explain, to some degree, the systematic error of the TLC system.

Institute of Analytical Chemistry,
University of Stockholm, Roslagsvägen 90,
S-104 05, Stockholm 50 (Sweden)

LARS ERIK STRÖMBERG
GUNNAR WIDMARK

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Detection of carboxylic acids on thin-layer chromatograms by their reaction with iodide–iodate and amylose

Selective detection reagents provide a useful adjunct to R_F data in identification of compounds separated by thin-layer chromatography. Although a number of reagents for visualizing carboxylic acids have been reported[1–7], interference by residual solvents often limits their sensitivity. This report describes a spray reagent composed of iodide, iodate and amylose which can be used to detect carboxylic acids on silica gel or cellulose after chromatographic separation in acid or basic systems.

Iodide is oxidized rapidly and quantitatively by iodate in the presence of acid by the reaction,

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$

NOVAK AND DLASK[8] exploited this reaction for detecting acids on paper chromatograms after development in chloroform–acetone–water–formic acid or ethanol–ammonia–water systems. Chromatograms from the acid system were dried for 16 h at room temperature, and the acids were immediately visible as brown spots after spraying with a mixture of potassium iodide, potassium iodate and starch. The papers developed in the basic system were dried for 1 h at room temperature; brown spots developed 1–2 h after spraying. Their reagent contained about 0.01% starch, equivalent to about 0.002% amylose. It has been pointed out[9] that it is the amylose content of starch which affords the characteristic intense blue color of the starch–iodide complex. The reagent described here contains 0.33% amylose. This may account for differences in the results observed. The higher concentration of amylose affords visualization of the acids as blue spots which appear almost immediately after development in either acid or basic systems.

Procedure

Test solutions of each acid were spotted in 10-μl volumes of 5 mg/ml acetone solutions 3 cm from the bottom of 20 × 20 cm Analtech Uniplates®, which consisted