

Spectrophotometric determination of polycyclic aromatic hydrocarbons separated by thin-layer chromatography, and evaluation of the light sensitivity of hydrocarbon spots

Thin-layer chromatography has proved valuable as a tool for the separation and identification of the components of complex mixtures of polycyclic aromatic hydrocarbons¹⁻⁷. The quantitative determination of the hydrocarbons in the spots is of considerable interest and some methods, based on extraction of the spots followed by spectrophotometric determination, have been suggested^{5,8} as well as the measurement of the spot area³.

Following our recent study⁷ on the separation of a number of polycyclic aromatic hydrocarbons, including separation on silica gel plates impregnated with caffeine, we aimed at examining the possibility of determining, quantitatively, the hydrocarbons separated on such plates by a simple extraction-absorption spectrophotometric technique. The hydrocarbons used were: anthracene, pyrene, 3:4-benzopyrene, perylene, 1:12-benzoperylene and chrysene.

The absorption of caffeine restricts the useful wavelength region to above 300 m μ . Above this limit the ultraviolet absorption spectra of equimolar solutions of pyrene in absolute ethanol and in this solvent saturated with caffeine were found to be identical with respect to both wavelength and intensity of absorption maxima. It was concluded therefore that caffeine, extracted together with a hydrocarbon, would not influence the absorption to be measured.

Interference might also be expected from pyridine, one of the constituents of the developer, which is also extracted and absorbs below 300 m μ . However, most of the pertinent aromatics have characteristic absorption above this point and it was found that this part of the absorption curves did not show dependence on added pyridine. In an experiment where pyrene was run alone on caffeine-impregnated plates with and without pyridine in the developer, recovery was found to be the same in each case.

It is an important point to remember that in thin-layer chromatography photo-oxidation of the adsorbed compounds may occur. Recently⁹ this fact was emphasized in the case of polycyclic aromatic hydrocarbons, the oxidation products of pyrene, for instance, having been identified and as a precaution against this it is necessary to run the chromatograms in the dark. During our previous investigation⁷, we observed that the hydrocarbon spots darkened at a much slower rate, and that the bright fluorescence remained constant for a much longer time on the caffeine-impregnated silica gel plates than on ordinary silica gel or alumina plates. An experiment illustrating these facts is recorded below.

Table I gives the results of a series of experiments in terms of per cent recovery of hydrocarbons extracted from the separate spots. Fig. 1 illustrates a typical chromatogram.

The order of magnitude chosen of the amounts of hydrocarbons spotted on the plate was relatively high. This was done in order to test the procedure for heavily loaded spots, since this makes the separation more difficult. Furthermore, it is easy to scale down, by a factor of about five, the amounts of hydrocarbons to be determined, by appropriate modifications of the extraction procedure and cell dimensions without changing the final concentration in the absorption cell. We modified the cell (rectangu-

TABLE I

PER CENT RECOVERY OF HYDROCARBONS FROM TLC-PLATES^a

In each of experiments 1-12 a spot containing anthracene, pyrene, 3:4-benzopyrene, perylene, 1:12-benzoperylene and chrysene^b was developed by two-dimensional chromatography, three runs in each direction. Detection by fluorescence in ultra-violet light.

Experiment No.	1 ^d	2	3 ^e	4	5	6	7	8 ^f	9	10	11	12 ^g
Hydrocarbon (λ ; ϵ) ^c	Amount (in γ) of hydrocarbon in spot ^h Per cent recovery of hydrocarbon											
Anthracene (356.5; 0.81 · 10 ⁴)	81								40.5			162
	60	82	94	94	90	88	93	70	77	94	108	87
Pyrene (319; 3.1 · 10 ⁴)	81								40.5			162
	62	80	95	105	95	100	100	98	72	105	94	80
3:4-Benzopyrene (384; 3.1 · 10 ⁴)	78								39			156
	65	55	71	90	64	65	69	60	85	77	62	83
Perylene (433; 3.76 · 10 ⁴)	20								10			40
	80	90	95	80	100	97	97	85	100	105	90	83
1:12-Benzoperylene (383.5; 2.7 · 10 ⁴)	21								10.5			42
	approx.			75 ⁱ					56			67

^a Plates: Silica gel, 0.6 mm, impregnated with caffeine (1 g per plate).

Developer: Light petroleum with 4% of pyridine.

^b For chrysene, see text.

^c Wave-length (λ , in $m\mu$) and molar extinction coefficient (ϵ).

^d 5% Pyridine in the developer.

^e Four runs in the 1st direction.

^f The plate, after developing and drying, was kept unprotected in the laboratory for 4 days before the spots were extracted.

^g The adsorbent layer was 1 mm thick, containing 1.7 g of caffeine.

^h In all of the experiments 1-8 the spots were identically loaded.

The same applies to experiments 9-11.

ⁱ The absorption curves did not allow exact readings.

lar, light path 1 cm) by inserting a loosely fitting piece of teflon in it with a 3 mm wide passage for the light. Thus, the volume of solution necessary was reduced to 1 ml. Depending on the hydrocarbon at hand, the concentration can be reduced so that even smaller amounts are determinable, as can be seen from the results with perylene.

Among the hydrocarbons examined only chrysene absorbs too weakly to be determined under the given conditions. 1:12-Benzoperylene was used in amounts comparable to those for perylene, but, although a reasonable recovery could be obtained, the uncertainty is considerable. Recovery of anthracene, pyrene, and perylene was in most cases better than 80-90%, whereas results were poorer and varied more for 3:4-benzopyrene. One reason for this is that the 3:4-benzopyrene partly invades the perylene spot and that perylene has a weak absorption at the wavelength used for determination of 3:4-benzopyrene, thus making the evaluation of the absorption peaks rather uncertain.

Extractions with 5 and 3 ml of ethanol, respectively, were performed on plates having smaller amounts of pyrene and 3:4-benzopyrene, so that the final concen-

tration of the extract is comparable to that in experiments 9–11 in Table I, *viz.* 3–4 γ /ml. Recovery was 75 and 40 %, respectively, indicating that the general extraction procedure (using 10 ml of ethanol) cannot be reduced to below 5 ml without depreciating the results. In one instance (expt. 8) waiting for four days before scraping off and extracting the spots did not alter the results much.

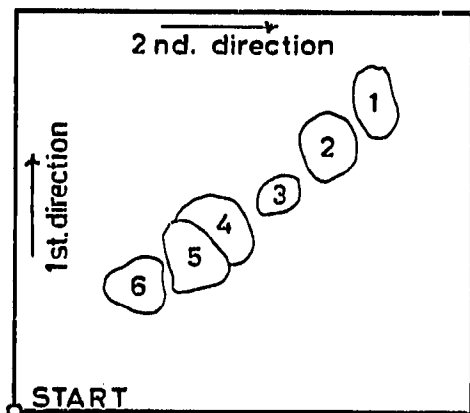


Fig. 1. Development in two directions for the separation of the following six hydrocarbons: (1) anthracene; (2) pyrene; (3) chrysene; (4) 3:4-benzopyrene; (5) perylene; (6) 1:12-benzoperylene.

The general procedure to be described was used to determine the amounts of the five above-mentioned hydrocarbons in a specimen of coal tar. The results (see below) agree well with published values¹⁰.

Experimental

The procedure for preparing and developing plates has been described before⁷. For details of plates and single runs, see Table I.

The spots were scraped off and extracted three times for 10 min with 3 ml each of absolute ethanol in a test tube immersed in a water bath. It is important that thorough agitation (performed with a glass rod) be maintained, otherwise the extraction will not be complete. This treatment was followed by centrifuging and decanting the solution. After cooling, the volume was made up to 10 ml. The absorption curves were determined on a Perkin Elmer 137 U.V. spectrophotometer.

Effect of caffeine and pyridine. The negative influence of these compounds on the absorption curves above 300 $m\mu$ was shown as described above. With special reference to the extraction procedure an experiment was performed as follows: ten spots, five containing 51 γ and the other five containing 102 γ of pyrene, were applied to three caffeine-impregnated silica gel plates and run with light petroleum without added pyridine. This experiment was repeated with 4 % of pyridine in the solvent. The amount of pyrene in each spot was determined using the general procedure set out above. Recovery of pyrene in the two experiments was $89 \pm 4\%$ and $90 \pm 4\%$ respectively.

Effect of prolonged exposure to light on the spots. A caffeine-impregnated silica gel plate (0.3 mm) was spotted with the six hydrocarbons and treated in the usual way⁷. Development with light petroleum-pyridine was always performed in a darkened room. Afterwards the plate was exposed to air and daylight (January) for several

days and repeatedly examined in ultraviolet light (360 m μ). Simultaneously an ordinary silica gel plate and an alumina plate were treated in the same way. On the two latter plates, after a relatively short time (1–2 h), the spots darkened and the fluorescence weakened (anthracene and pyrene) or almost disappeared (3:4-benzopyrene, perylene, 1:12-benzoperylene and chrysene), while on the caffeine-impregnated plate they were completely unchanged after four days. The fluorescent spots developed from a coal tar sample on each of the three plates behaved in a similar manner to the corresponding spots of the pure compounds.

Examination of coal tar. The tar was an untreated sample. A solution was made up of 59 mg of the tar in 1 ml of benzene and a total of 80 μ l was spotted on the plate in ten spots. On another plate a single spot of the tar was run together with reference substances. From the positions and fluorescence colours of the spots after five runs, and from absorption spectra of the extracted spots the following hydrocarbons were identified (figures in brackets state amounts of the hydrocarbons found, as per cent of weight of tar): anthracene (0.21), pyrene (0.58), 3:4-benzopyrene (0.19), perylene (0.03) and 1:12-benzoperylene (0.12).

Acknowledgement

We wish to thank the Statens almindelige Videnskabsfond, Denmark, for a grant.

*Department of Chemistry, University of Aarhus,
Aarhus (Denmark)*

JØRGEN LAM
ARNE BERG

- 1 TH. WIELAND, G. LÜBEN AND H. DETERMANN, *Experientia*, 18 (1962) 430.
- 2 N. KUCHARCZYK, J. FOHL AND J. VYMĚTAL, *J. Chromatog.*, 11 (1963) 55.
- 3 H. MATSUSHITA, Y. SUZUKI AND H. SAKABE, *Bull. Chem. Soc., Japan*, 36 (1963) 1371.
- 4 M. FRANCK-NEUMANN AND P. JÖSSANG, *J. Chromatog.*, 14 (1964) 280.
- 5 E. SAWICKI, T. W. STANLEY, W. C. ELBERT AND J. D. PFAFF, *Anal. Chem.*, 36 (1964) 497.
- 6 E. SAWICKI, T. W. STANLEY, J. D. PFAFF AND W. C. ELBERT, *Chemist-Analyst*, 53 (1964) 6.
- 7 A. BERG AND J. LAM, *J. Chromatog.*, 16 (1964) 157.
- 8 J. SCHMELTZ, R. L. STEDMAN AND W. J. CHAMBERLAIN, *Anal. Chem.*, 36 (1964) 2499.
- 9 M. N. INSCOE, *Anal. Chem.*, 36 (1964) 2505.
- 10 *Elsevier's Encyclopaedia of Organic Chemistry*, Elsevier Publishing Company, Amsterdam.

Received March 29th, 1965

J. Chromatog., 20 (1965) 168–171

Separation of imidazoles by cellulose thin-layer chromatography

In a previous communication¹ the separation of imidazoles on alumina and silica thin-layer chromatoplates is described. The use of thin-layer cellulose provides a useful supplement to this technique, particularly for the separation of imidazole-carboxylic acids.

Compact spots are obtained in acidic solvents but some tailing is evident with basic solvent systems. As with thin-layer chromatography of imidazoles on alumina,

J. Chromatog., 20 (1965) 171–173