COLUMN CHROMATOGRAPHY AND SPECTROSCOPY IN THE ANALYSIS OF AIRBORNE POLYCYCLICS

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INTRODUCTION

The measurement of polycyclic hydrocarbons in air samples, for some fifteen years, has been based mainly on ultraviolet absorption measurements made after chromatographic separations of air sample extracts on alumina (or silica gel) columns. The work of WEDGWOOD AND COOPER[1], COOPER[2] and COMMINS[3] may be cited as representative. The heights of characteristic ultraviolet peaks have been measured above a somewhat arbitrary base line[2,3] and the peak heights found have been interpreted as proportional to the concentration of a particular hydrocarbon. Measurements may be made on individual chromatographic fractions and the individual values combined to give the total for a particular hydrocarbon. For sensitivity reasons, it may be necessary to combine fractions and reduce the volume of the composite fraction so that the concentration will be high enough to be analysable by ultraviolet absorption. Originally, ultraviolet measurements were made by manual spectrophotometers. A great deal of the tedium associated with manual measurement has been eliminated by the now widespread use of ultraviolet recording spectrophotometers. These instruments are, in fact, commonly used as both the monitoring and measuring instruments. Similarly, recording fluorimeters are now coming into use, and with their sensitivity so much greater than that obtainable from ultraviolet instruments, eluate fractions, "empty" to ultraviolet, prove to contain measurable amounts of polycyclics by fluorescence. It is worthy of note that as early as 1943, WEIL-MALHERBE[4] worked out the chromatography of benzo[*a*]pyrene (B*a*P) on alumina and silica gel, using a home-made fluorescence instrument. Fourteen years later in 1957, CAHNMANN[5] using a recording ultraviolet spectrophotometer, established the behaviour of benzo[*a*]pyrene on deactivated silica gel. It would be almost unthinkable now, to do column chromatography without the aid of some kind of automatic recording spectroscopy.

An integrated approach is illustrated by gas chromatography where, under ideal conditions, the air sample extract may be separated into a series of individual peaks which can be measured on the recorder chart chromatogram. DUPIRE[6] separated and measured tar oil fractions by gas chromatography. LIBERTI *et al.*[7] measured polycyclic hydrocarbons in three samples of dust by gas chromatography. Their method involves concentration of 100 ml of cyclohexane extract to 5 ml, partition of the polycyclics between solvents and final concentration of sample, under reduced pressure, to about 10 μl. They obtain beautiful chromatograms, but

from our experience, we would expect almost total loss of anthracene and phenanthrene, and loss of a great deal of both pyrene and fluoranthene during the concentration process. This concentration step is presumably necessary on sensitivity grounds. Similar high evaporative losses of anthracene, phenanthrene, pyrene and fluoranthene are cited by GRIMMER AND HILDEBRANDT[8]. WILMSHURST[9] analysed polynuclear arenes by gas chromatography after going through a conventional chromatographic step "to concentrate the polynuclear arenes", and also perhaps to clean up, or lower the background of the sample before gas chromatographic analysis.

WILMSHURST admits there may be some ambiguity connected with some of the peaks found. As he says "chrysene and benz[*a*]anthracene... were detectable subsequent to the peak". DE MAIO AND CORN[10] measured polycyclics in ten composite samples of Pittsburgh air, but again found it necessary to concentrate the benzene extract to attain a concentration which was usable with the gas chromatograph.

It must be pointed out here that it is quite feasible to analyse polycyclics in prepared extracts by fluorescence, without concentration. In fact, it is usually desirable to dilute and re-analyse at lower concentrations to check that concentration quenching is not at work. The sensitivity of ultraviolet is certainly less than fluorescence and it is sometimes necessary to concentrate to get a usable response. An illustration of this is the measurement of perylene in air samples where perylene structure may not appear in the spectrum until the extract has been concentrated[11]. In the gas chromatographic work cited, concentration was used by all workers. A rough comparison of the monitoring sensitivities would be fluorescence, ultraviolet and gas chromatography, in that order.

The best analytical approach would be to combine gas and liquid column chromatography with fluorescence and ultraviolet instrumentation. For example, unlike liquid chromatography, gas chromatography separates benzo[*k*]fluoranthene (B*k*F) cleanly from benzo[*a*]pyrene, but it does not separate B*a*P from benzo[*e*]-pyrene (B*e*P). Provided B*k*F is absent, it is possible, however, to measure B*a*P by fluorescence and B*e*P by ultraviolet in an eluate containing both[11]. B*a*P and B*k*F can both be measured by fluorescence in an extract or eluate containing a mixture of these two[12]. Efforts are being made to combine the best features of all, but for the present, gas chromatography will not be further discussed, and the emphasis will be on the older liquid column chromatography with spectroscopic identification and measurement of compounds.

The sampling and preparation history of an air sample has perhaps as much to do with the final total accuracy of the values obtained as the actual analytical method. For example, the solvent used to extract the air sample is not a matter of indifference. Extraction should be rapid and complete and substances interfering with the subsequent analysis should not be extracted, ideally. This optimum situation has not yet been achieved, and in the following actual sampling errors and losses will not be discussed.

Several practical analytical difficulties which are encountered are lack of sufficient instrumental sensitivity, apparent losses of hydrocarbon during chromatography and the background. These problems will now be considered.

REAGENTS AND APPARATUS

Alumina adsorbent

Type H 100–200 mesh of Peter Spence is used without any washing treatment. It is heated overnight at 145° and deactivated by the addition of 1.8 % water.

Cyclohexane

Technical grade cyclohexane of British Drug Houses is percolated through a bed of active carbon, Pittsburgh Chemical BPL 12 X 30. The product obtained should show less than 0.5 p.p.h.b. fluorescence calculated as quinine base.

Ether

Ethyl ether, fluorimetric grade from Hartmann-Leddon, Philadelphia, Pa., is used without further treatment.

Chromatographic columns

A glass tube 1.0 cm I.D. and 40.0 cm long is fitted with a teflon plug stopcock. The column is filled to a depth of 12 cm with a slurry of the deactivated alumina in cyclohexane.

Instrumentation

For ultraviolet absorption a Bausch & Lomb spectrophotometer No. 502 with fixed slits was used, as well as a Cary 14 recording spectrophotometer. For fluorimetric measurements a modified Aminco-Bowman spectrophotometer was used with a 1 P28 photomultiplier tube and slit arrangement No. 2.

EXPERIMENTAL

Before doing any experiments we can theorise, knowing the systems we are dealing with, that apparent low recovery of polycyclics may be caused by one or a combination of the following:

(1) Lack of sensitivity of the measuring technique,
(2) Irreversible adsorption on the column,
(3) Tailing on the column,
(4) Incomplete column separations,
(5) Decomposition on the column.

Each of these possibilities was investigated separately and appropriate experiments were performed to see which of these premises might be true.

With any instrumental technique used to measure consecutive eluates, a certain minimum concentration is necessary to get a measurable response. For concentrations below this limit a zero value is obtained. With a detection limit of 0.010 absorbance units we see from Table I that to detect B*a*P by ultraviolet absorption we require a concentration of 0.7 μg per 1 ml, or in a cuvette of 3 ml volume, a total amount of 2.1 μg. This is based on measurement at the characteristic B*a*P peak at 401–403 nm. It is interesting to note that precisely the most important hydrocarbon in the group, B*a*P, has the least favourable detection limit. On the other hand, B*a*P is a strong fluorescence emitter and is therefore well suited for measure-

TABLE I

CONCENTRATIONS DETECTABLE BY ULTRAVIOLET MEASUREMENT
For detection limit of 0.010 absorbance units.

Hydrocarbon	*Concentration*		*Wavelength (nm)*
	μg/ml	*μg/3 ml*	
Pyrene	0.04	0.12	325–340
Coronene	0.06	0.18	335–342
Fluoranthene	0.05	0.15	284–290
Benzo[*e*]pyrene	0.075	0.22	323–337
Anthanthrene	0.04	0.12	422–430
Benzo[*g,h,i*]perylene	0.01	0.03	376–393
Benzo[*k*]fluoranthene	0.10	0.30	395–405
Benzo[*a*]pyrene	0.70	2.1	397–406

ment by fluorescence[12]. Comparing sensitivities available from ultraviolet and fluorescence for B*k*F ultraviolet measurements may be made over a range of 0 to 10 μg/ml and fluorescence measurements over a range of 0–0.1 μg/ml. Rated conservatively, there is a sensitivity differential of more than 100 times in favour of fluorescence measurements. A practical illustration of the difference in usable sensitivity is shown by Fig. 1, in which B*a*P was measured in the same eluate fraction of an air sample by both techniques. An easily measured response is given by fluorescence but ultraviolet absorption shows nothing measurable. It is easy to see that the use of the wrong measuring technique might give the false impression that B*a*P was absent. Apparent low analytical recoveries may also be explained as due to the remaining four factors. Since these factors are all column-dependent, they were investigated together.

Fifty micrograms of ten different polycyclics were chromatographed separately on the alumina column previously described, using cyclohexane as eluting agent. Quantitative measurements were made on the eluates by ultraviolet or fluorescence,

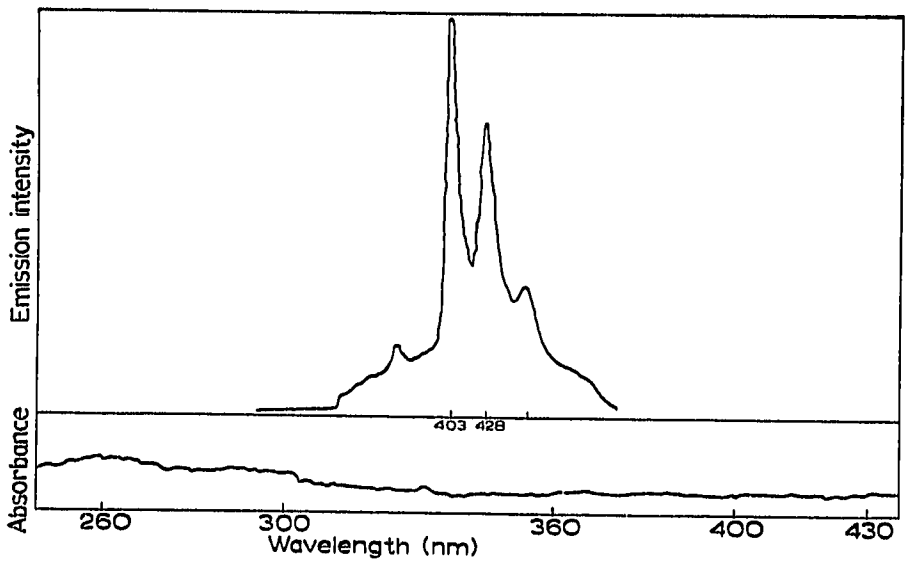

Fig. 1. Practical comparison of fluorescence and ultraviolet sensitivities based on same sample.

depending upon the concentration of a particular hydrocarbon in the eluate. All the eluates containing a particular compound were combined, a quantitative measurement was made and this was compared with the value given by the same amount of the same standard not chromatographed. Table II gives the percentage recovery for 50 μg of each of the ten polycyclic hydrocarbons listed. Within experimental error,

TABLE II

RECOVERY FROM THE COLUMN

No.	*Compound*	*μg found*		*Recovery (%)*	
		U.V.	*Fluorescence*	*U.V.*	*Fluorescence*
1	Naphthalene	50.8	—	101.5	—
2	Fluorene	49.6	—	99.3	—
3	Phenanthrene	51.2	—	102.5	—
4	Anthracene	49.9	—	99.9	—
5	Pyrene	49.6	51.2	99.2	102.3
6	Fluoranthene	48.3	49.2	96.6	98.4
7	Triphenylene	49.9	48.4	99.8	97.1
8	Chrysene	—	47.0	—	94.0
9	Benz[*a*]anthracene	—	47.0	—	94.0
10	Benzo[*a*]pyrene	—	52.5	—	105.0

recovery is complete and it is clear that there is no decomposition on the column, and that the adsorption process is reversible for the particular column conditions used. It is to be noted that the separations were performed using only cyclohexane. If a more polar solvent such as ether were used in admixture we should certainly still recover all the polycyclic.

To see whether the cause of apparent low recovery might be incomplete separation or tailing, the ten polycyclics were chromatographed together on an alumina column. Cyclohexane and increasing quantities of ethyl ether were used to elute as shown in Table III.

TABLE III

TYPICAL ELUTION

Volume (ml)	*Solvent*
0-315	Pure cyclohexane
315-415	0.5 % ether
415-470	1.0 % ether
470-690	1.5 % ether
690-740	2.0 % ether
740-840	3.0 % ether
840-860	4.0 % ether

Each eluate fraction was scanned in the ultraviolet. The chromatogram obtained is shown in Fig. 2. The shape of the curves indicates that tailing is negligible for all practical purposes. (See also chromatograms of GRIMMER AND HILDEBRANDT[8].) It is interesting to note the poor separation of the two groups, fluorene, phenanthrene and anthracene and triphenylene, benz[*a*]anthracene and chrysene. Meanwhile

Table IV shows that recovery is fairly good at the wavelengths used for "base line" measurements. Recovery varies from a low of 83 % for fluorene to a high of 110 % for phenanthrene. As can be seen, incomplete separation does not explain low recovery because the effect can be either positive or negative. Considering standards only, singly or in mixture no evidence has been found for low recoveries or column losses. Standard solutions, of course, lack the background which is present in an air sample.

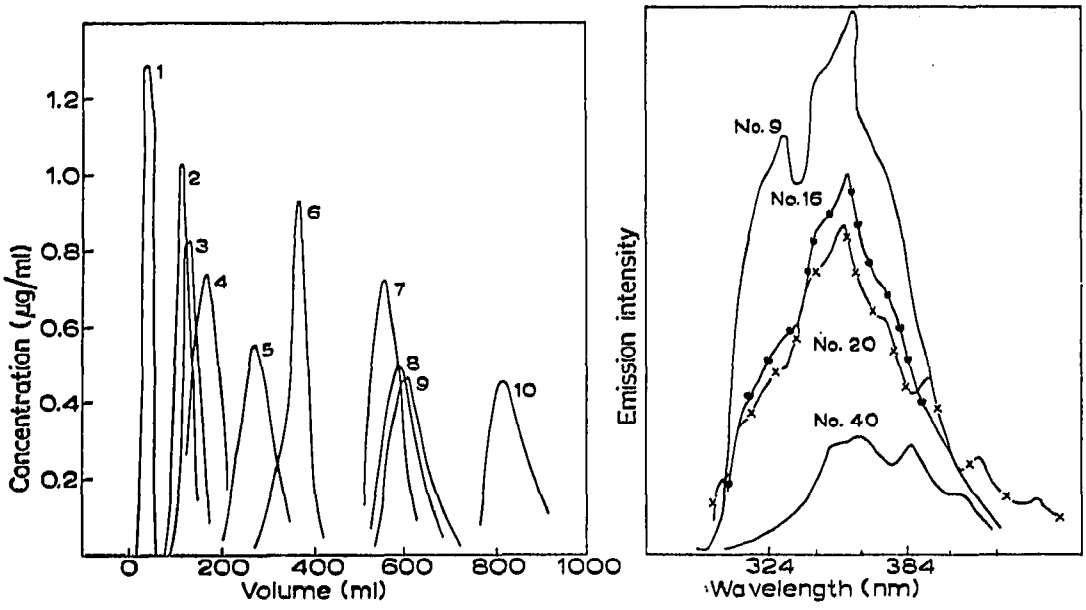

Fig. 2. Chromatogram of standard mixture of ten polycyclic hydrocarbons.

Fig. 3. Variation in compound X with chromatographic development.

Apparent recovery losses which cannot now be attributed to the column might, for the sake of argument, be attributed to the hiding of peaks by the background, leading to negative measurement errors. Accordingly, an air sample extract was chromatographed on an alumina column and fluorescence emission spectra were

TABLE IV

SEPARATION OF A MIXTURE

No.	*Compound*	*Found*	*U.V. recovery (%)**	*Wavelength (nm)*
1	Naphthalene	51.5	103.0	
2	Fluorene	41.5	83.0	296–305
3	Phenanthrene	54.8	109.7	287–301
4	Anthracene	50.9	101.9	371–381
5	Pyrene	46.6	93.2	325–340
6	Fluoranthene	48.9	97.9	284–290
7	Triphenylene	52.1	104.2	245–252
8	Benz[*a*]anthracene	47.3	94.6	283–293
9	Chrysene	46.8	93.6	263–273
10	Benzo[*a*]pyrene	47.3	94.7	375–390

* Mean recovery 98.6 %.

recorded for each eluate fraction using 289 nm as the exciting wavelength. This wavelength was chosen for three reasons: (a) it is a good excitation wavelength for the material which seems to be the cause of the background; (b) most of the aromatics present should be excited at this wavelength; and (c) this wavelength is less subject to concentration interference than wavelengths in the 200–270 nm region. It was soon discovered that each eluate apparently contained varying amounts of the same compound "X" as may be seen from Fig. 3. The concentration of X starts at a maximum decreasing continuously with successive eluate fractions. The predominant peak at 360 was preceded by small peaks as shown by fraction 9. In fraction 20, the peak at 330 has almost disappeared, the intensity at 360 has dropped sharply and at least three new peaks have appeared at wavelengths above 360. At no time, in these experiments, were spectra for known pure compounds obtained. The peak at 360 nm was always present. From these results the background might be described as:

(1) One compound emitting at 360 present in much higher concentration than any of the others,

(2) A mixture of hydrocarbons having a common structure like derivatives of naphthalene or phenanthrene,

(3) Overloading of the column.

Using gas chromatography on a silicone oil column at 225° with a hydrogen flame detector, it was found that gas chromatograms of the aromatic fraction of an air sample extract showed numerous peaks, each component present in approximately equal amounts. This would seem to rule out No. 1 as a working hypothesis.

We already know from past experience that tailing on alumina of 1.8% water content is negligible for all practical purposes. Nor can incomplete separation of the polycyclics explain the background effect. Exciting the ten component standard mixture at 289 we find that there is no peak at 360 nm in the emission spectrum.

This leaves us with the last premise that the background may be due to column overloading. Results obtained by fluorescence measurements on air sample eluates indicate that the spectrum of the so-called background is almost identical to the spectrum of an air sample extract *not* chromatographed. Moreover a curve of log background against elution volume has the shape of a typical decay curve. Fluorescence measurements suggest that there is no separation of this background material before 400 ml. On the other hand, we know from ultraviolet measurements that a separation of fluoranthene and pyrene is already taking place. The curve of log fluorescence intensity *versus* eluate volume is identical to a curve of 1/carbon number *versus* log retention volume. Lastly the shapes of the curves vary depending upon the exciting wavelength, see Fig. 4. This is an indication that the absorption is different and that a separation could be taking place, since as the emission wavelength is increased, the size of the molecule becomes bigger.

CONCLUSIONS

The use of fluorescence is mandatory in the measurement of polycyclic hydrocarbons in air samples. Without its use, the analyst would be seriously handicapped with regard to sensitivity. With respect to the special problem of B*a*P analysis, this is best carried out by fluorescence rather than ultraviolet absorption[12,13]. From our

experience we feel that much of the data on B*a*P-in-air concentrations in the literature may be seriously in error. Before the mutual interference of B*a*P and B*k*F were pointed out by SAWICKI published measurements probably reflect B*k*F as well as B*a*P[14,15].

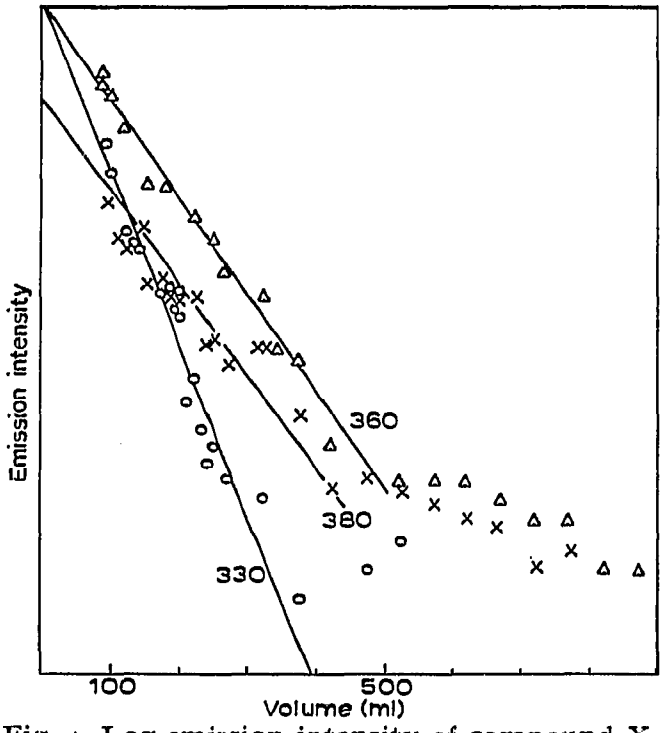

Fig. 4. Log emission intensity of compound X *versus* eluate volume for various emission wavelengths.

We can see no evidence for losses on the chromatographic column and accordingly no need to correct for such losses. It is possible that apparent losses may be due to interference from the background. Our present feeling is that the background may be due to overloading of the column or, on occasion, to incomplete separation of a mixture of hydrocarbons having a common structure. These hydrocarbons are likely to be of the two to three ring type. It must be remembered that overloading of the column and incomplete separation are different effects, but the influence on the chromatogram will be the same. A permanent condition is the fact that the aromatic fraction being analysed is only a very small portion of the total air sample. Our column is considerably shorter than that used by CLEARY[16], with equally good separations, more quickly obtained.

In air sample analysis two interfering effects may be due to the background. Firstly, certain peaks are certainly obscured, which limits the certainty of identification. Secondly, the values obtained must be less than the true value if we exclude the possibility of other interferences. Work is in progress to identify the background and to evaluate the degree of analytical error associated with it.

SUMMARY

In the authors' experience, no column losses occur during the liquid column chromatography of polycyclic hydrocarbons as found in air samples.

Monitoring of the numerous column eluates is most rapidly performed by recording instruments based on ultraviolet or fluorescence. These instrumental techniques complement one another but fluorescence is far superior in sensitivity. In the special problem of the analysis of benzo[*a*]pyrene, fluorescence must be used since ultraviolet results are quite unreliable.

The constant presence of an unknown material, compound X, has been demonstrated in air samples. This may be a lubricating oil residue derived from automobile exhaust.

Work is in progress to identify the "background" and to assess its effect in air sample analysis.

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