THE ROUTINE DETERMINATION OF POLYCYCLIC HYDROCARBONS IN AIRBORNE POLLUTANTS

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INTRODUCTION

There are no easy methods for the analysis of polycyclic hydrocarbons found in polluted air and tobacco tars. A certain degree of complexity and a considerable amount of labor is inherent in all these methods and the accuracy is not very high. In the following we present a method which we have used routinely for some years. Care is necessary and complexity is still present, but the separations and analytical accuracy have been improved.

APPARATUS AND MATERIALS

A Soxhlet extractor of 300 ml capacity is required. Chromatographic columns are prepared by joining a stopcock with 1 mm bore to a 25 cm length of glass tubing of 1 cm internal diameter. A fraction collector may be used to collect chromatographic fractions overnight. However, we have found that it is quite convenient to run succeeding fractions on a recording spectrophotometer immediately after each fraction is eluted from the column. Evaporation of the sample extract or of an aliquot may be accomplished by using a vacuum oven or by the use of clean dry air or nitrogen.

Spectrophotometer

A ratio recording spectrophotometer is a necessity because of the large number of chromatographic fractions which have to be analysed. The useful range of the instrument used in these studies is from 2200 Å to 4500 Å with a resolution of 1 to 2 Å (Cary 14 of Applied Physics Corp.).

Fluorimeter

The analysis of benzo[*a*]pyrene (BaP) in the presence of benzo[*k*]fluoranthene (BkF) is readily accomplished by using fluorescence techniques[1]. An instrument capable of providing the necessary selection of excitation wavelengths is required. The instrument must also be able to scan the emission spectrum. Such an instrument has been developed by a suitable modification of an Aminco-Bowman fluorimeter[2].

Cuvettes

The cells used for ultraviolet absorption studies may be of the conventional rectangular fused silica design with a 1 cm light path. For fluorescence measurements

similar cells, polished on all four sides, are used. These fluorescence cells may also be used for ultraviolet absorption measurements.

Alumina

The alumina used is type H, 100–200 mesh, of Peter Spence, England. This alumina is slightly alkaline. We have found that it is unnecessary to prewash this alumina before use. A quantity of alumina, perhaps 300 g, is activated by placing in an oven overnight at 140°. About 100 g of such activated alumina is removed from the oven, cooled, weighed and placed in a flask having a ground glass stopper. The alumina is covered with sufficient cyclohexane to give a supernatant depth of perhaps 0.5 in. Water equal in weight to about 1.8 % of the alumina is added to the alumina–cyclohexane slowly and the mixture is shaken thoroughly. Because water and cyclohexane are immiscible, the water is evenly dispersed throughout the alumina, assuring uniform deactivation. The alumina is then equilibrated by allowing to stand overnight. Methods of determining the activity of deactivated alumina have been suggested by COMMINS[3] and GRIMMER AND HILDEBRANDT[4].

Silica gel

Material of 28–200 mesh (Davison Co., Baltimore) is kept in an oven at 140° and removed for preliminary separations if required[5].

Cyclohexane

Fluorimetric or spectro grade cyclohexane may be purchased from a number of suppliers. Benzene may be present in large amounts in some spectro grades of cyclohexane. In view of the large quantities of cyclohexane which may be used it is worthwhile preparing this in the laboratory. A suitable starting material is the technical grade supplied by British Drug Houses which is free from aromatics. This is purified by percolation through a glass column 2.5 in. in outer diameter, 18 in. in length and provided with a "teflon" plug stopcock. This is packed with activated carbon (Pittsburgh Chemical Co., 12 × 30 mesh) supported on a glass wool plug. The cyclohexane produced is free from ultraviolet absorbing and fluorescing impurities to better than 0.5 p.p.h.b., calculated as quinine base.

Iso-octane

This spectro grade solvent is purified in the same way as the cyclohexane.

Ethyl ether and benzene

The fluorimetric grade available from Hartmann-Leddon, Philadelphia, is satisfactory. These solvents could be purified in the laboratory but the small volumes used suggest purchase rather than preparation.

Polycyclic aromatic hydrocarbons

Standards of the various hydrocarbons of interest are available from a number of sources in North America and Europe. Zone refined quality is preferred if available. The purity of such standards must be confirmed before use by all available techniques such as chromatography, melting point, ultraviolet absorption, fluorescence and anything else which may be of help.

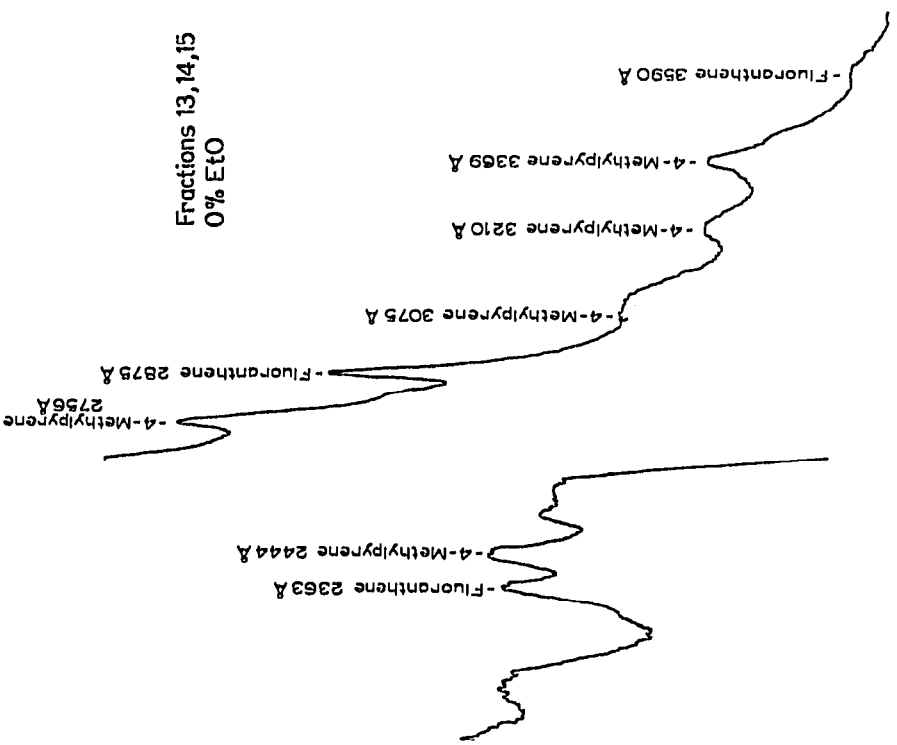

Fig. 2. Pyrene, fluoranthene and 4-methylpyrene in fractions 13–15.

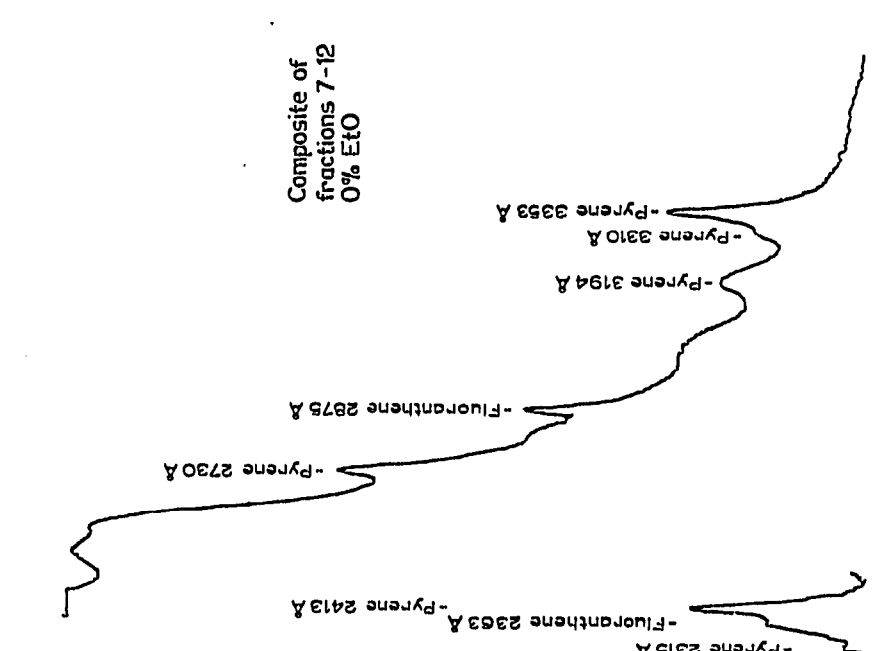

Fig. 1. Pyrene and fluoranthene in combined fractions 7–12.

The glass fiber sheets, on which air samples are usually taken, may be extracted *in toto* or aliquot circles may be punched from the sheet. The whole sheets, or discs cut from them, are placed in a Soxhlet and extracted with cyclohexane for 24 h. The total cyclohexane extract is filtered to remove carbon and glass fibre, made up to a standard volume and stored in the refrigerator until required.

METHOD

The cyclohexane extractable content of the total extract is established by carefully evaporating a 10 ml aliquot. A measured portion of the total cyclohexane extract, sufficient to contain 15–25 mg of cyclohexane-extractable material is measured into a weighed 5 ml beaker, carefully reduced in volume and then diluted with iso-octane. The solution is refluxed carefully in the beaker under a watch glass to remove the remaining cyclohexane and it is then reduced to 2 to 3 ml and placed on a column of activated silica gel[5]. Elution with iso-octane is continued until 400 ml have passed through the column. The aromatic fraction left on the silica gel column is then removed with 100 ml of benzene. This "aromatic fraction" in benzene is evaporated carefully to dryness using filtered air or nitrogen and taken up in 4 to 5 ml of cyclohexane. The concentrate is carefully poured onto the alumina column and allowed to pass almost entirely into the alumina. The column is then capped with 0.5 cm of deactivated alumina and elution with pure cyclohexane is begun. Approximately 70 chromatographic fractions of 8 ml each are collected in clean centrifuge or test tubes. Elution is begun using pure cyclohexane. Nothing appears in the first 6 fractions. Pyrene and fluoranthene may be seen in fractions 7 to 12 (Fig. 1). 4-Methylpyrene is found in fractions 13, 14 and 15, in addition to pyrene and fluoranthene (Fig. 2). In the fractions 16 to 34, benz[*a*]anthracene, and chrysene appear. The decline of chrysene structure at wavelengths 2682 Å and 2579 Å is the signal for changing the eluting solvent from pure cyclohexane to a mixture of 2 % ether in cyclohexane. The first fraction (35) associated with this change is shown in Fig. 3. This more polar mixed solvent will elute benzo[*e*]pyrene (BeP), BaP, BkF and benzo[*g,h,i*]perylene (BghiP) in 20 to 25 fractions. The first three of this group are shown in fraction No. 45 (Fig. 4). After concentration by evaporation, two peaks for perylene may be seen in the same fraction (Fig. 5). This illustrates one of the problems with ultraviolet absorption as a monitoring procedure, namely, the possibility of failing to see certain components originally present in low concentration in the fractions, or whose absorbing effect is low, with accompanying low sensitivity. When the BghiP structure at peaks 3625 Å and 3840 Å begins to decline, the eluting solvent is changed to 4 % ether in cyclohexane and anthanthrene and later coronene begin to show. Fraction 62 (Fig. 6) shows 4 characteristic peaks for anthanthrene. Coronene is eluted last, over a group of 18 fractions, one of which, No. 69, is illustrated (Fig. 7). Seven rather good peaks for coronene are shown.

Due to the variable composition of air samples, it is possible that there may be some variability in the optimum location for changing solvent polarity. For this reason, the operator should follow the elution process by observing the appearance and disappearance of characteristic peaks, changing solvent polarity when this is appropriate.

The fractions taken from the column may be weighed and the polycyclic con-

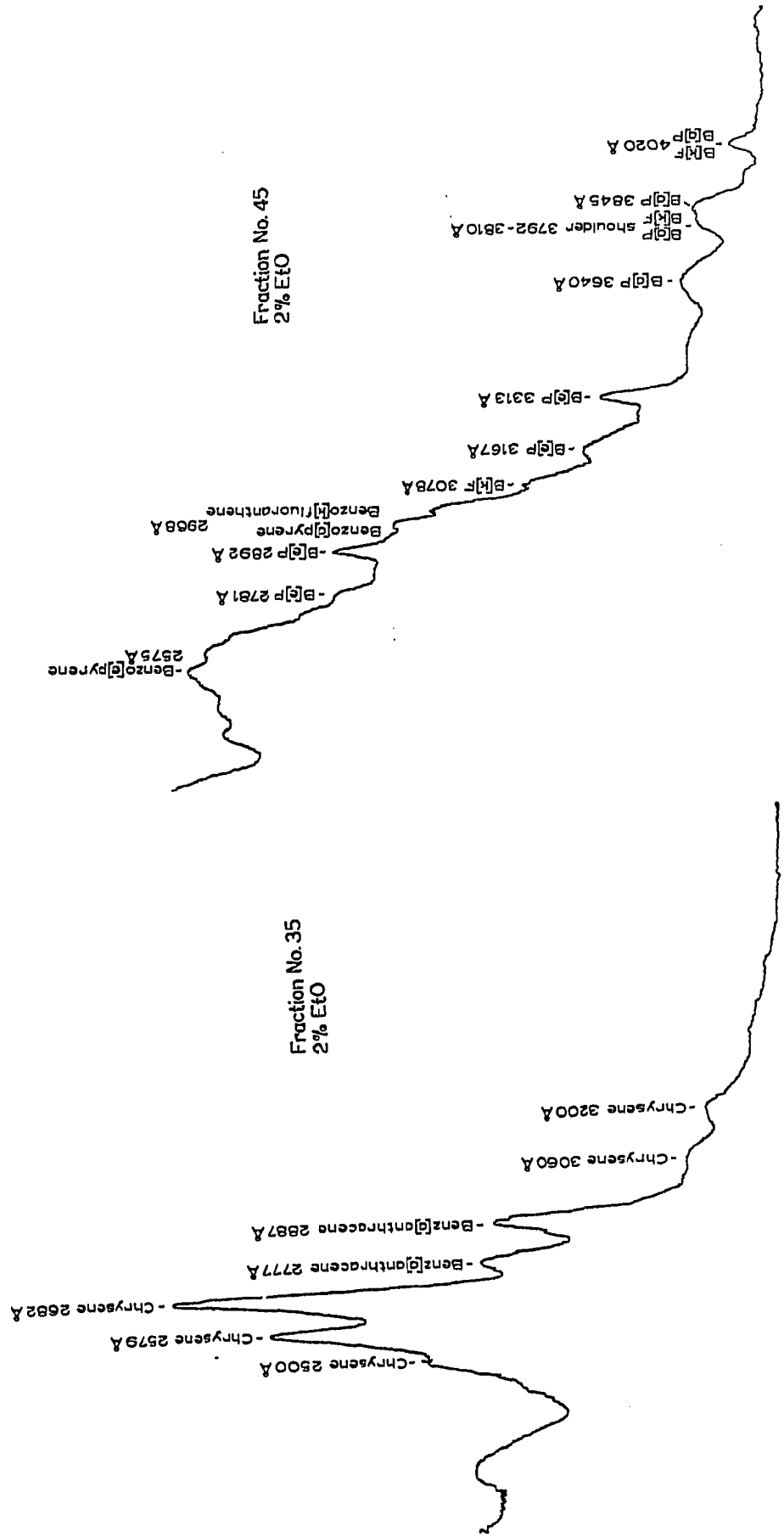

Fig. 4. Benzo[*a*]pyrene, benzo[*e*]pyrene and benzo[*k*]fluoranthene, fraction 45.

Fig. 3. Benz[*a*]anthracene and chrysene, fraction 35.

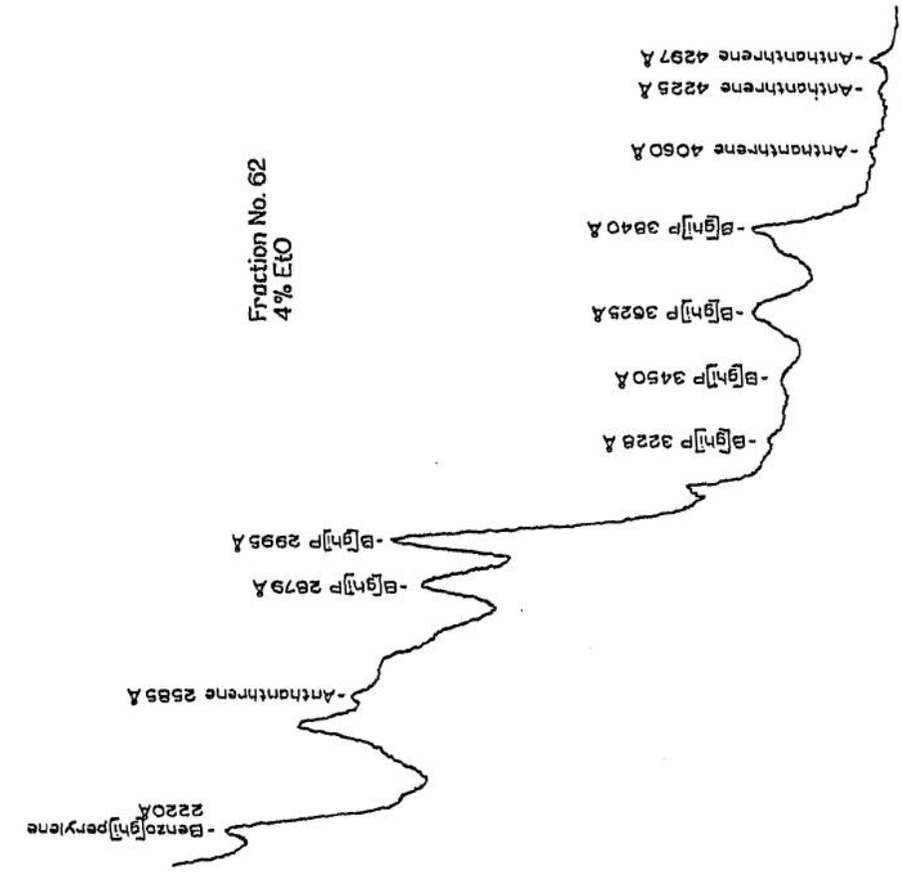

Fig. 6. Benzo[*ghi*]perylene and anthanthrene, fraction 62.

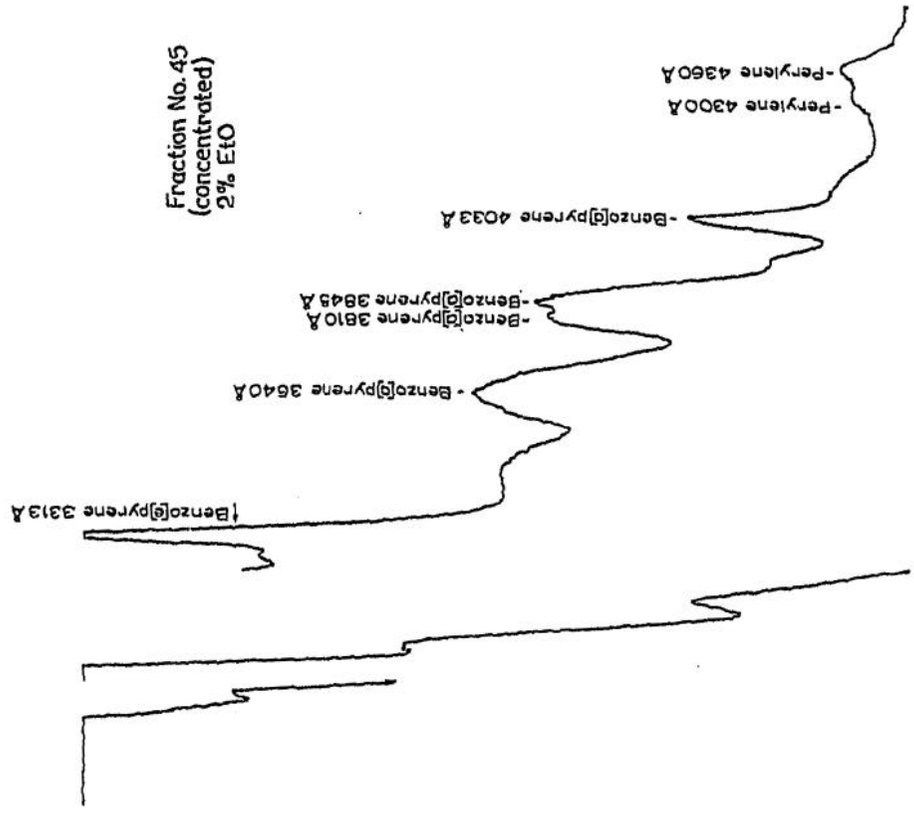

Fig. 5. Perylene appearing in fraction 45 after concentration.

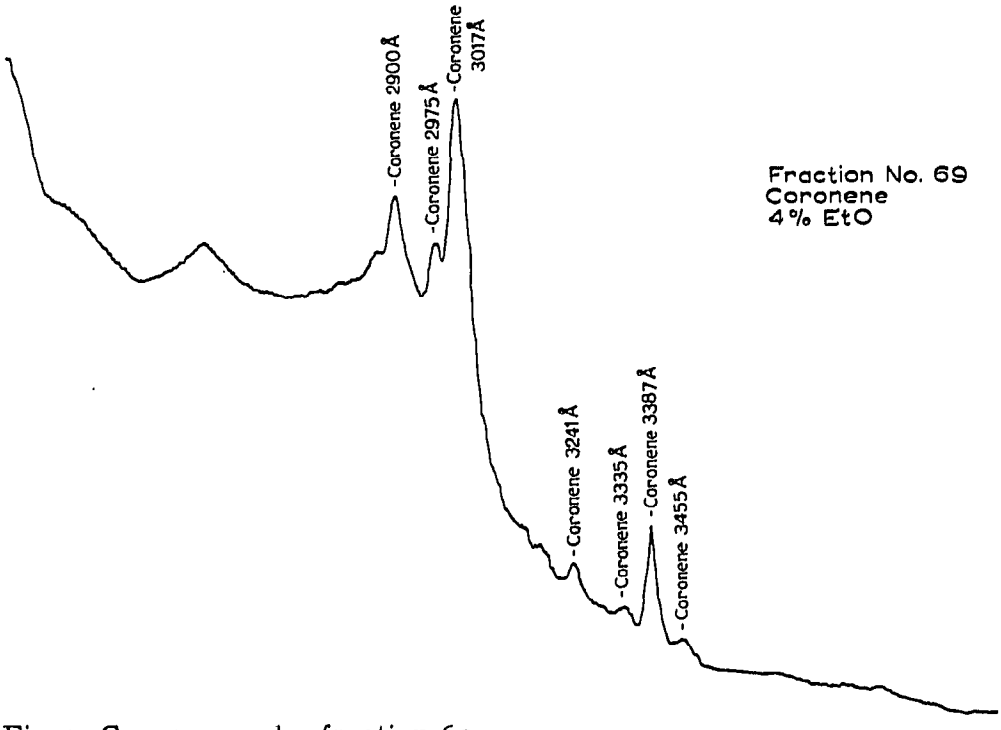

Fig. 7. Coronene only, fraction 69.

centrations measured fraction by fraction, or the fractions containing a particular polycyclic may be combined, concentrated and the total concentration of the polycyclic determined with one ultraviolet measurement. The second procedure is very useful in many cases where the concentration of aromatics is very low. The fractions containing BeP, BaP and BkF are grouped together but handled in a different manner. The BeP concentration is measured by ultraviolet absorption in the usual way. The solution is now diluted ten to one hundred fold to bring the concentration within the linear range for fluorescence. With the emission monochromator set at 4030 Å the solution is excited at 3070 Å and 3850 Å and two fluorescence emission values are obtained from which the individual concentration of BaP and BkF are determined[1].

DISCUSSION

In order to be of maximum value the spectra obtained must provide both qualitative and quantitative information. Whether a compound can be identified within a mixture of unknown composition will depend largely on two factors:

(1) The identity, or otherwise, of the band wavelengths of the unknown with the bands of known standard solutions.

(2) The shapes and ratios of the various peaks shown in the unknown and standard spectra.

Ideally, each of the bands displayed by the standard should also be identifiable in the unknown sample. Background and other interference frequently prevent the appearance of all bands. Consequently then, only the more intense bands are regularly available for identification comparisons. Much of the useful structure of air-

borne polycyclics lies between 2400 Å and 3000 Å. Unfortunately, some aliphatic hydrocarbons and other so far unidentified "background" materials also have adsorption in this region. Particular attention must therefore be devoted at all times to minimising interferences of this kind. Prior separations on silica gel are intended to isolate the aromatics from the aliphatic groups. The use of ultra pure solvents is mandatory. The amount of sample placed on the chromatographic column must, as a minimum, strike a balance between the sensitivity afforded by the analytical technique but the amount, as a maximum, must not overload the column. Since all identification and concentration measurements are made by comparison with standard curves prepared using pure compounds, the greatest accuracy will be obtained from measurements made on spectra which resemble as closely as possible the standard curves for the pure substance. The greater the departure of the structure of the unknown sample from that of the standard the greater the inherent error of any quantitative measurement.

As may be seen from the spectrum of the composite, formed from fractions 7 to 12 and that of 13 to 15, subtle changes occur in the structure of the absorption bands, which if not noticed, could lead to mistakes both in identification and measurement. In composite Fig. 1 the pyrene bands at 3353, 3294, 2730 and 2413 indicate unequivocally the presence of pyrene. In the composite Fig. 2 these bands have shifted to 3369, 3210, 2756 and 2444. This is a maximum change of 84 Å and therefore easily overlooked. The 4-methylpyrene identified in fractions 13 to 15 is a common and important interference in the accurate measurement of pyrene. In completely analogous fashion alkyl derivatives of fluoranthene and chrysene can and do display similar upscale shifts which can cause both qualitative and quantitative errors.

We do not wish to suggest that the method outlined is the only way to analyse polycyclic hydrocarbons in air samples. It is, however, a method with which much experience has been obtained, the separations are sharper than most we have seen, the separation sequences have been unvarying, and the solute elution positions have, so far, been accurate to several ml. For the most accurate work only the very best instrumentation can be permitted. The wavelength scale of the ultraviolet instrument used in this work is actually accurate to 2 Å. The wavelength scale of our modified fluorimeter is probably not accurate to better than 2 Å although the "instrumental" reproducibility is good to 1 Å or 2 Å. The complete analysis of a prepared air sample extract, including chromatography measurements by ultraviolet or fluorescence and calculations requires 2 days.

Ultraviolet and fluorescence measurements have, until now, been made by the "base line" method of COMMINS[6] although we are investigating other methods of peak measurement which may be better. In addition to the actual technique of peak measurement, work is proceeding to identify the "background" if possible and thus to compensate for possible analytical errors due to this.

There are some important differences when one compares the method outlined with other apparently rather similar methods in the literature. We endeavour to ensure uniform deactivation of the adsorbents. It is not possible to perform a practically useful chromatographic separation if the activity of the adsorbent is too high[7]. The use of ether to increase the polarity of the eluting solvent is an expedient only, designed to hasten the chromatographic process. This it does, but at the expense

of muddying the separations. It is desirable, therefore, if ether is to be used, to restrict the proportions to a bare minimum. Ether may also react with the hydrocarbons[7]. We have not encountered column losses in chromatographic separations and do not have to make a correction for such column losses[3,8]. Such losses, if they occur, could quite conceivably be due to the use of overactive adsorbents. Our alumina is slightly alkaline in reaction, that used by others tends to be acidic. We do not feel that the long column used by CLEARY[9] has any advantage since the elution time is greatly increased without the separation being noticeably better.

Much of the tabulated data for BaP in the literature is probably inaccurate on several counts. Let us consider only the question of measuring BaP and/or BkF in mixture. If ultraviolet measurements are used exclusively on such fractions, the results are likely to be poor if the measurements are based on the 401 peak of BaP. This must be particularly true of ultraviolet measurements made before the interference of BkF was pointed out[8]. For this reason, we measure the BaP and BkF by fluorescence[1]. CLEARY does not seem to have found BkF, which we invariably find in the BaP fraction[8].

A chromatographic separation is inherent in the method described. In addition to "background" and "peak" measurements we are also investigating the possible and probable errors in polycyclic analysis individually and as they affect the final result.

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SUMMARY

The chromatography of polycyclic hydrocarbons from air samples is a combination of science and art. The art is to achieve the best usable separations in the shortest working time. This involves compromises including the activity of the alumina, the depth of the adsorbent and the amount of ether, or other polar solvent used.

Ultraviolet spectroscopy as a monitoring technique is not sufficiently sensitive. This lack of sensitivity may be the reason that column losses have been reported. Fluorescence as a monitoring technique is much more sensitive and must be used when benzo[*a*]pyrene is being measured, since the benzo[*k*]fluoranthene present in the benzo[*a*]pyrene fractions causes serious interference with measurements made at the characteristic benzo[*a*]pyrene peak at *ca.* 402 nm.

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