DIRECT FLUOROMETRIC SCANNING OF THIN-LAYER CHROMATOGRAMS AND ITS APPLICATION TO AIR POLLUTION STUDIES

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INTRODUCTION

Direct scanning of chromatographic fractions has been used extensively in gas chromatographic analysis. The direct scanning of paper chromatograms for quantitative estimations of various compounds has been reviewed recently[1]. Instruments used routinely to scan paper chromatograms can be used to scan thin-layer plates. Several techniques for the rapid scanning of radioactivity on thin-layer plates have been described[2,3]. Spots on a thin-layer chromatogram have been treated with an appropriate reagent and the resultant colors measured with a suitable scanning photometer[4]. Recently the photometric estimation of fluorescent spots on thin-layer chromatograms has been described[5,6].

This paper presents a direct method for automatic fluorometric scanning of thin-layer chromatograms with much greater selectivity and sensitivity than has hitherto been possible. By use of the proper excitation and emission spectral bands, a chromatogram can be examined for an individual compound or for a family of compounds. In addition, quenchofluorometric techniques applied to the thin-layer plate eliminate many interferences and further improve the sensitivity.

EXPERIMENTAL

Equipment

An Aminco-Bowman spectrophotofluorometer was used with the following settings: sensitivity, 50; meter multiplier, 0.1 or 0.3; slit arrangement No. 2; and phototube, RCA type 1P21. The instrument was equipped with an Aminco automatic scanning attachment for paper or thin-layer chromatograms. The scans were recorded on a Minneapolis-Honeywell Recorder Model No. Y143X(58)-(VB).

Strips approximately 0.5 in. wide containing the origin and the sub-spots are scanned at appropriate excitation and emission wavelengths. The chromatogram can be on plastic sheet or thin glass so that it can be cut into narrow strips after development[7]. Cellulose and cellulose acetate adsorbents can be peeled from ordinary glass plates with the help of a chromatographic preserving medium[8] (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, New York) and then the strips can be examined fluorometrically.

RESULTS

A mixture of five polynuclear aza heterocyclic compounds was separated by thin-layer chromatography; the chromatogram was then treated with trifluoroacetic acid fumes and scanned (Fig. 1). An excitation and an emission wavelength were selected to give bands for all the compounds. By selection of appropriate excitation

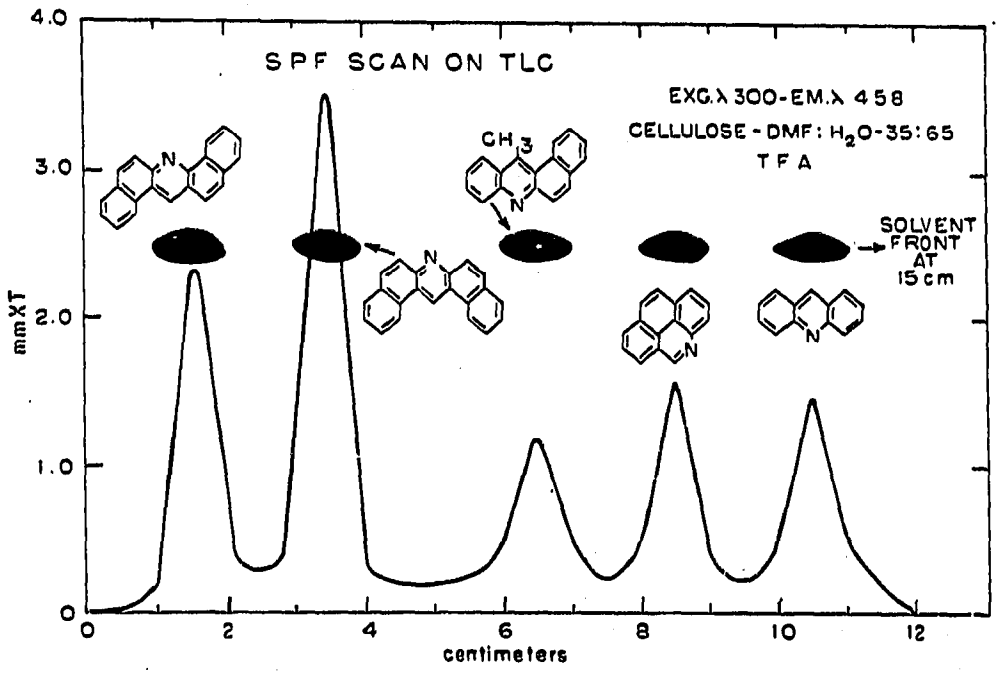


Fig. 1. Spectrophotofluorometric scan of a mixture containing 0.1 μg each of dibenz(a,h)acridine, dibenz(a,j)acridine, 12-methyl-benz(a)acridine, benzo(l,m,n)phenanthridine, and acridine.

and emission wavelengths the compounds could be readily differentiated and characterized (Fig. 2). Thus, at excitation wavelength 402 mμ and emission wavelength 440 mμ only the dibenzacridines gave bands. Dibenz(a,h)acridine was readily characterized through the presence of the prominent band obtained with excitation wavelength 310 mμ and emission wavelength 447 mμ. In this fashion the dibenzacridines are readily characterized by the scanning bands obtained with the appropriate excitation and emission wavelengths at the R_F value of the compounds. In the same way the other compounds were characterized by scanning bands. In addition, the emission spectrum was obtained at the scanning excitation wavelength and the excitation spectrum at the scanning emission wavelength. These spectra could be obtained by the operator[7] after the scan or could be done automatically. The R_F value, the scan band, the excitation and emission wavelengths at which the band was obtained, and the fluorescence excitation and emission spectra were obtained quickly and directly from the thin-layer chromatogram. Characterization was performed readily and quickly.

An example of the characterization of polynuclear aromatic hydrocarbons is shown in Fig. 3. A mixture of benzo(a)pyrene, benzo(e)pyrene, benzo(k)fluoranthene, and perylene was separated on cellulose acetate with ethanol–toluene–water (17:4:4, v/v), and then scanned at the appropriate wavelengths. Benzo(a)pyrene, benzo(k)-

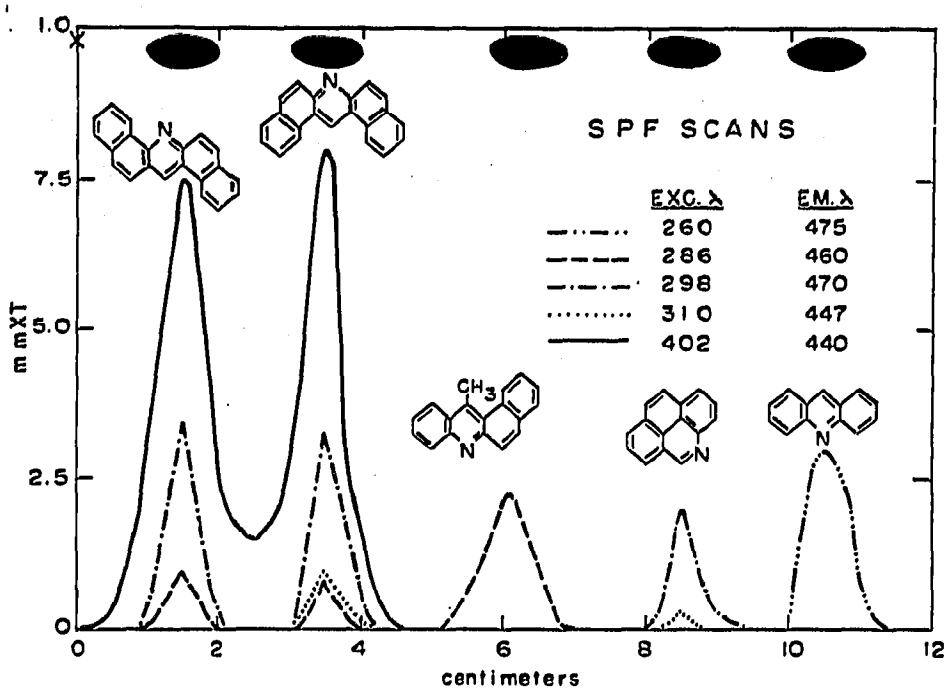


Fig. 2. Scan of the same mixture at various excitation and emission wavelengths.

fluoranthene, and perylene were readily characterized by their R_F values and the positions of their scan bands. The areas under the bands can be used to estimate the quantities of these hydrocarbons. An example of the linear relationship between the area under the band and the amount of benzo(*a*)pyrene in the spot on the thin-layer plate is shown in Fig. 4. Each point on the figure is the average of 11 values. The range of these values is shown by the length of the small perpendicular line passing

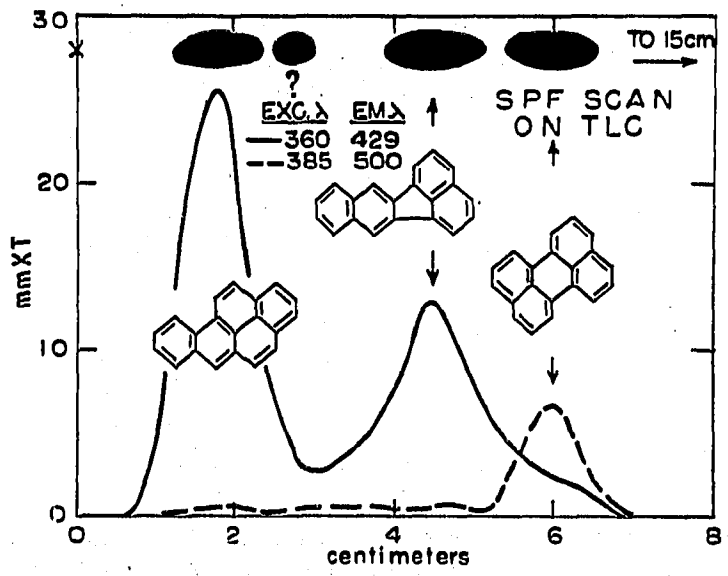


Fig. 3. Spectrophotofluorometric scan of a mixture containing 0.1 μg each of benzo(*a*)pyrene, benzo(*k*)fluoranthene, perylene and benzo(*a*)pyrene. Mixture had been separated by cellulose thin-layer chromatography with ethanol–toluene–water (17:4:4, v/v) as developing solvent.

through the point. With more than 60 μg of benzo(a)pyrene the slope of the area–concentration line becomes less steep up to 200 μg of benzo(a)pyrene.

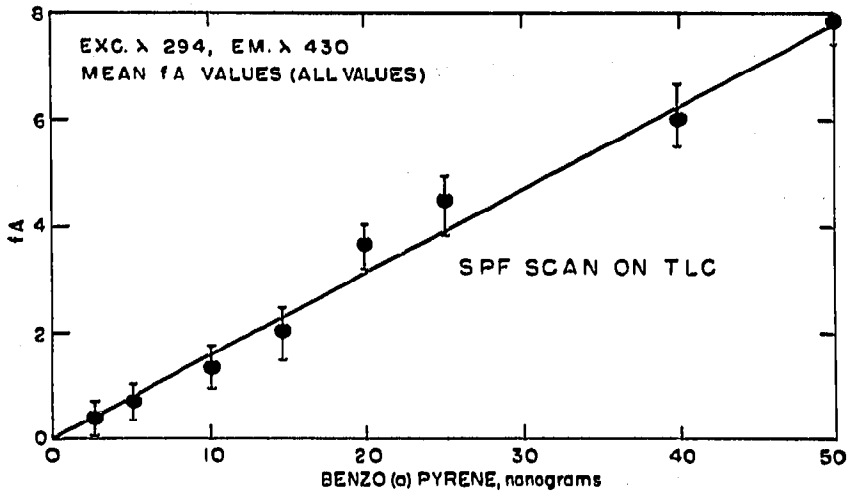


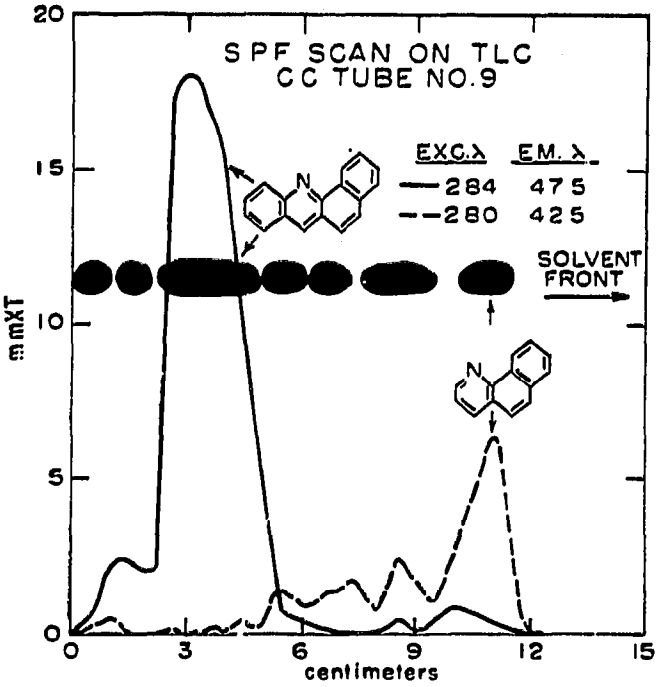
Fig. 4. Spectrophotofluorometric scan on a cellulose acetate thin-layer chromatogram. Area of band *versus* nanograms of benzo(a)pyrene.

APPLICATION

The scanning technique worked well with particulates obtained from the air and from air pollution source effluents. An atmospheric sample whose main contaminant was coal-tar-pitch fumes was selected for study because its basic fraction was much more complicated than any other samples we have studied. The basic fraction was separated on an alumina column and the benz(c)acridine subfraction was then further separated with cellulose thin-layer chromatography[9]. The chromatogram was scanned at the excitation and emission wavelengths that would give the best response for benz(c)acridine and benzo(h)quinoline (Fig. 5). Both of these compounds were characterized by their R_F values and the scan band. In addition, direct spectrofluorometric examination of the spots characterized the compounds more fully through the excitation and emission spectra of their salts. An alumina column chromatographic fraction whose absorption spectrum in pentane showed weak bands at 371 and 391 mμ was separated by means of cellulose thin-layer chromatography. A fluorometric scan showed the presence of dibenz(a,h)acridine (Fig. 6). For the estimation of these compounds, standards (0.001 to 1 μg) must be examined on the same plate.

For routine assay of polynuclear compounds by the scan technique to be practical, the technique must provide definite advantages over the column chromatographic–ultraviolet absorption spectral procedures presently used[10]. The investigator should be able to analyze smaller samples in less time; for this reason the column chromatographic step would have to be omitted. Although two-dimensional thin-layer chromatography followed by the fluorometric scan procedure shows definite promise for both assay and identification, much more work must be done before the method can be used in routine assay.

An alumina column-chromatographic benzpyrene fraction from an urban atmospheric sample was separated on a cellulose acetate thin-layer plate (Fig. 7). Since this fraction was not extremely complicated, benzo(a)-pyrene and benzo(k)-



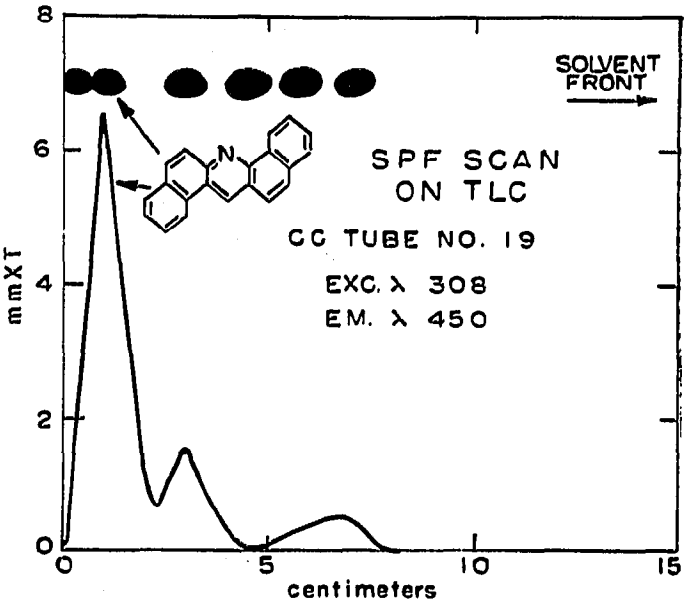
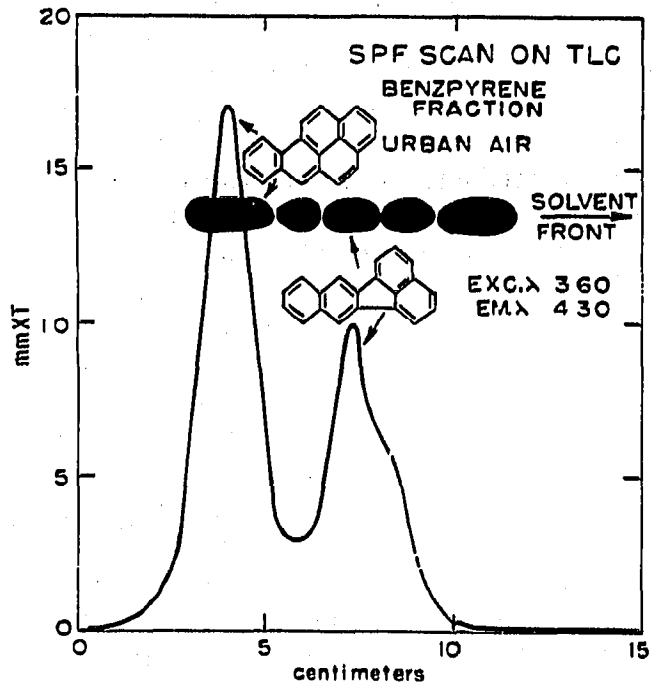


Fig. 5. Spectrophotofluorometric scan of a benz(*c*)acridine fraction obtained from coal-tar-pitch polluted air. Previous to scan, sample was separated by cellulose thin-layer chromatography with dimethylformamide–water (35:65) as developer. Spots were treated with trifluoroacetic acid fumes before scan.

Fig. 6. Spectrophotofluorometric scan of the dibenz(*a*,*h*)acridine fraction (as in Fig. 5).



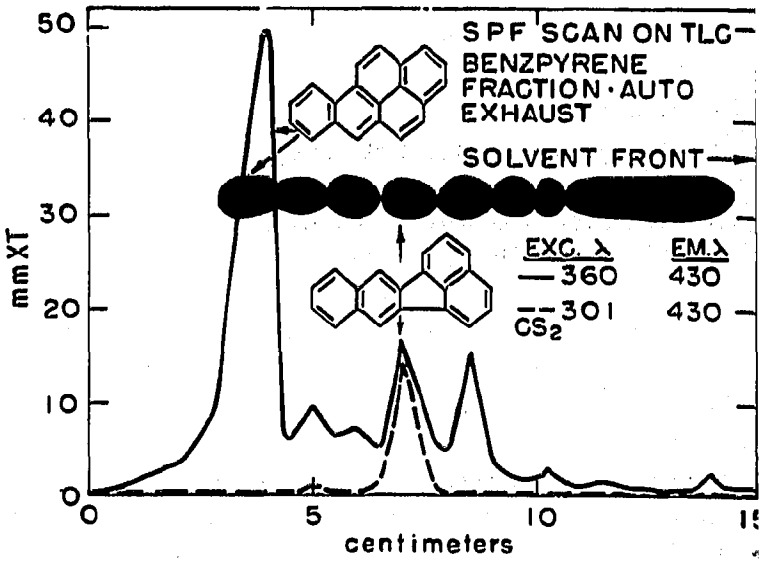


Fig. 7. Spectrophotofluorometric scan of an atmospheric benzpyrene fraction. Previous to scan, sample was separated by cellulose acetate thin-layer chromatography as in Fig. 3.

Fig. 8. Spectrophotofluorometric scan of the benzpyrene fraction obtained from auto exhaust fumes. Separation as in Fig. 3.

fluoranthene were readily characterized on the plate. Although the analysis of solutions through ultraviolet absorption spectrophotometry is much more accurate, this method cannot be used for determining the nanogram to microgram amounts that can be estimated by the fluorometric scan method.

In the scan of Fig. 7 the benzo(*k*)fluoranthene band showed a shoulder derived from some unknown compound. This band was resolved with the help of the quenching effect[11], as shown for the more complicated benzpyrene fraction obtained from auto exhaust (Fig. 8). With the help of carbon disulfide the fluorescences of all the components (except benzo(*k*)fluoranthene) of this chromatogram (or of the one shown in Fig. 7) were quenched. Use of the appropriate excitation and emission wavelengths was also of value here. Fig. 8 shows the potentiality of quenchofluorometry in the scanning of fluorescent molecules.

SUMMARY

Fluorometric scanning of thin-layer chromatograms was investigated. The method worked well for many compounds in nanogram to microgram amounts. By the described scanning technique, fluorescent compounds were quickly characterized by the presence of a scan band obtained with the appropriate excitation and emission wavelengths at the R_F value of the compound. Further evidence was obtained from the emission and excitation spectra at the scanning wavelengths. With these techniques and fluorescence quenching, selectivity of the fluorometric method was improved considerably. With the scan method, estimation techniques were found to be feasible. The methods were shown to be of value in air pollution analysis.

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