

# THE VARIOUS QUENCHING EFFECTS IN THIN-LAYER CHROMATOGRAPHY

## APPLICATION TO AIR POLLUTION

E. SAWICKI AND H. JOHNSON

*Laboratory of Engineering and Physical Sciences, Division of Air Pollution, Robert A. Taft Sanitary Engineering Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio (U.S.A.)*

(Received November 29th, 1965)

### INTRODUCTION

Quenching effects on phosphorescent<sup>1,2</sup> or fluorescent<sup>3</sup> reagents have been useful in locating non-fluorescing ultraviolet-absorbing chemicals on thin-layer chromatograms.

Recently a few selective quenching reagents used in the developer or after development were introduced for the location and preliminary characterization of fluorescent compounds containing various types of functional groups<sup>4</sup>. These techniques are useful primarily in the analysis of fluorescent compounds. We wish to emphasize that the procedure could be applied also to the direct analysis of non-fluorescent compounds by chemically changing these latter compounds on the plate into fluorescent derivatives. The synthesis of fluorescent derivatives on the plate followed by direct fluorometric examination is readily performed, as shown in recent work<sup>5,6</sup>.

In this paper we discuss the ramifications and possibilities of fluorescence quenching in thin-layer chromatographic analysis. Examples are given to show the potentiality of this technique.

### EXPERIMENTAL\*

#### *Chemicals and equipment*

All solvents were distilled before use. All polynuclear compounds were obtained pure and were recrystallized to a constant melting point when chromatography showed that they were impure. An Aminco-Bowman Spectrophotofluorometer with a solid-state attachment was used in the direct fluorometric examination of thin-layer chromatograms.

#### *Chromatography*

A mixture of 20 g alumina and 10 g 2,4-dinitrophenoxy starch (Anheuser-Busch, Inc., St. Louis, Mo.) in 96 ml of absolute methanol was blended for 1/2 min. Five

\* Mention of commercial products does not constitute endorsement by the Public Health Service.

plates were coated to a thickness of 500  $\mu$ . They were then air dried and were ready for use in an hour. In the separation the solvent front reached 5 cm in 10 min, 10 cm in 30 min, and 15 cm in 60 min. Separation on alumina took twice as long.

#### DISCUSSION AND RESULTS

Eight main types of quenching techniques have been used to some extent in thin-layer chromatographic analysis. The most common one involves chromatography on an adsorbent in which a fluorescent or phosphorescent compound is evenly dispersed<sup>7</sup>. Rhodamine B, fluorescein, and fluoranthene have been used as the fluorescent compound; inorganic phosphors of various types have been used as the phosphorescent compound. Under ultraviolet light ultraviolet-absorbing compounds are seen as dull to black spots against the fluorescent background.

In the second technique the developed chromatogram is sprayed (or dipped) with a solution of the fluorescent or phosphorescent material<sup>8</sup>. The ultraviolet-absorbing materials are then readily located under the ultraviolet light as dark non-fluorescing spots.

A third quenching technique involves the use of a fluorescent screen to locate an ultraviolet-absorbing spot<sup>9</sup>.

A fourth type of quenching effect that has proved valuable in thin-layer chromatography is obtained with the use of a volatile quencher in the developing solvent<sup>4, 10</sup>. Volatile quenchers that can be used are diacetyl, carbon disulfide, isopropylamine, 2-butanone, trifluoroacetic acid, trifluoroacetic anhydride, nitrogen dioxide, nitromethane, and pyrrole. The boiling points of the volatile quenchers range from about 30 to 100°.

With pentane plus 2-nitropropane as a developing solvent fluoranthenic hydrocarbons can be distinguished from other types of polynuclear aromatic hydrocarbons. While the plate is wet with the nitroalkane, the non-fluoranthenic hydrocarbons do not fluoresce while the fluoranthenic ones do. After the plate has dried, all the hydrocarbons fluoresce.

A fifth type of quenching effect involves the use of a nonvolatile quencher in the developer<sup>4</sup>. Pentane-nitrobenzene (9:1) has been used. Such a compound quenches the fluorescence of the aromatic hydrocarbons while other types of compounds fluoresce. The concentration of nitrobenzene in the developer can be adjusted so as to quench almost all fluorescent compounds. Another type of quenching agent could be used so that the polynuclear aromatic hydrocarbons would fluoresce while the fluorescences of other types would be quenched; e.g. with N,N-dimethylaniline, the fluorescence of the aza heterocyclic hydrocarbons would be quenched while the fluorescence of many of the aromatic hydrocarbons would be unaffected.

A sixth quenching effect is obtained when a developed chromatogram is sprayed, fumed, or treated with a volatile quencher<sup>4</sup>. By use of appropriate quenching gases a plate can be exposed to fumes in a closed container or each separate spot can be treated individually. Some of the volatile quenchers used in our laboratory are carbon disulfide, nitrogen dioxide, nitromethane, trifluoroacetic acid, alkyl nitrites, and alkyl nitrates. Nitrogen dioxide-trifluoroacetic acid (7:3, v/v) has been used successfully to differentiate aromatic hydrocarbons from aza heterocyclic hydrocarbons and to test for an aromatic hydrocarbon through its loss of fluorescence on treatment<sup>4</sup>.

TABLE I  
EFFECT OF QUENCHING AGENTS ON VARIOUS COMPOUNDS\*

Quencher**	8-Amino-fluoranthene	Benz(c)-acridine	7H-Benz-(d,e)-anthracen-7-one	Morin	Pyrene	Tryptophan
<i>Quencher in Al<sub>2</sub>O<sub>3</sub> adsorbent</i>						
1-Aminopyrene	Q → Q	Q → B	QO → Q	GO → B	EO → EO	EO → EO
Aniline	G → G	Q → B	QO → YO	GO → G	EO → EO	EO → EO
o-Cresol	G → IG	Q → Q	QO → YO	G → G	E → EO	E → E
Nitrobenzene	Q → Q	Q → G	EB → Y	G → G	Q → Q	E → E
1-Nitropyrene	Q → Q	Q → Q	QO → Q	G → G	Q → Q	EO → EO
Phenylhydrazine	E → G	Q → B	QO → YO	G → G	E → E	E → E
Phloroglucinol	G → BG	Q → BG	QO → Y	G → G	E → E	EB → E
Picric acid	Q → Q	Q → B	QO → Y	Q → G	Q → Q	EB → E
2,4-Dinitrophenoxy starch	Q → Q	Q → G	Q → Y	G → G	Q → Q	E → E
<i>Volatile quencher after development</i>						
N,N-Dimethylhydrazine	G	B	Q	G	B	fB
NO <sub>2</sub> -TFA (1:1)	Q	B	Y	Q	Q	Q
<i>Non-volatile quencher after development</i>						
Aniline	G	Q	Q	G	Q	Q
o-Cresol	G	Q	Q	G	EB	fB
Nitrobenzene	Q	Q	Q	Q	Q	Q
Pyrrole	G	Q	Q	G	Q	fB

\* B = blue, f = faint, G = green, l = light, O = orange, Q = quenched, Y = yellow, and → = fluorescence colors before and after trifluoroacetic acid treatment.

\*\* Liquids: 0.2 g per g alumina; solids: 0.1 g per g alumina; dinitrophenoxy starch-alumina (1:1).

The diverse results obtained with two types of volatile quenchers are shown in Table I for six fluorescent compounds, each containing a distinctive functional group. With this type of temporary quenching, compounds containing the functional group of interest could be located in a quick preliminary, nondestructive procedure.

The seventh type of quenching effect is obtained when the developed plate is treated with a nonvolatile quencher. Some useful quenchers are phenols, anilines, nitrobenzenes, nitrosobenzenes, hydrazines, benzaldehydes, quinones, polycyano compounds, and potassium permanganate. Much work needs to be done to investigate the ramifications and possibilities of this new type of functional group analysis.

If necessary, a strong quencher could be diluted to allow maximum use of its quenching ability. Such a quencher could be used at the highest concentration at which it would have a maximal effect on the interferences while the test substances remained fluorescent.

Dependent on the functional group of the test substance, a quencher can be used in alkaline or acidic solution to allow maximal use of its selective property. For example, the nonvolatile quenchers in Table I are best used for the analysis of the 8-aminofluoranthene and morin types of compounds. With the addition of acid to the quencher the fluorescence of most aromatic amines would be quenched while many of the polynuclear aza heterocyclic compounds would become fluorescent in acidic aniline and nitrobenzene.

The usefulness of the seventh technique is indicated by comparison of Figs. 1 and 2. The red visible spot, a natural internal marker, is valuable in comparing these

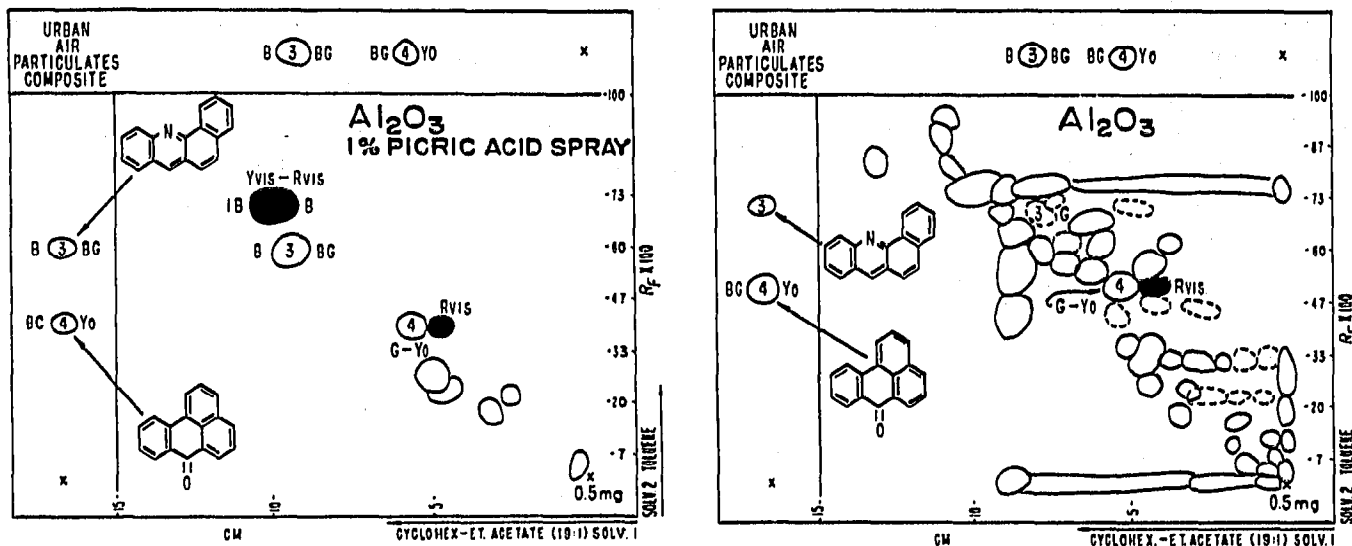


Fig. 1. Two-dimensional alumina thin-layer chromatogram of 0.5 mg of composite airborne particulate sample separated as in Fig. 2. Following development the plate is sprayed with 1% aqueous picric acid. Benz(c)acridine and 7H-benz(d,e)anthracen-7-one standards run one-dimensionally. Definitions as in Fig. 2.

Fig. 2. Two-dimensional alumina thin-layer chromatograms of 0.5 mg of the benzene extract of a composite of airborne particulate samples collected from about 100 American cities. Standards run one-dimensionally: (3) benz(c)acridine and (4) 7H-benz(d,e)anthracen-7-one. B = blue, G = green, l = light, O = orange, Y = yellow. Letters at left of spot represent fluorescence color on the wet chromatograms; letters at right, fluorescence colors after fuming with trifluoroacetic acid. Spots encircled by dashed line appeared only after fuming. Rvis = spot with red visible color.

figures (and also Fig. 3) since its location clarifies the relative positions of the various spots. Of all the spots originally present on this plate (similar to Fig. 2, a different run, which shows plate before treatment with quencher), only nine fluoresce after treatment of the plate with 1% picric acid spray (Fig. 1). Benz(c)acridine and 7H-benz(d,e)anthracen-7-one were readily characterized on the plate. The quenching treatment simplified the location and characterization of these compounds.

An eighth type of quenching effect is obtained through the use of an insoluble inorganic or organic compound as a quenching material in the adsorbent. Examples of some organic quenchers would be polymers or large insoluble compounds containing nitro, acetyl, phenolic hydroxyl, aromatic amino, hydrazino, polycyano, quinonic, or other appropriate groups. These types of quenchers produce a selective quenching effect with various families of fluorescent compounds and this provides a type of functional group analysis that can be performed directly on the thin-layer plate. This technique should allow the development of many new kinds of characterization and assay methods. The highly selective quenchofluorometric technique of analyzing for polynuclear hydrocarbons containing the fluoranthenic ring system in the presence of all other types of polynuclear aromatic hydrocarbons<sup>10</sup> could be developed for direct fluorometric assay on a thin-layer chromatographic plate.

The usefulness of this technique is shown in a comparison of Figs. 2 and 3. Both figures represent two-dimensional separation of the same benzene extract of a composite sample of urban airborne particulate (collected from approximately

100 large American cities). In the separation on alumina, Fig. 2, a large number of fluorescent spots were obtained. Also a large, vague fluorescent area was seen along both solvent fronts, but especially in the area diagonally opposite the origin. Benz(c)-acridine and 7H-benz(*d,e*)anthracen-7-one were characterized by direct fluorometric examination. In addition a red visible spot of unknown composition was readily noticeable.

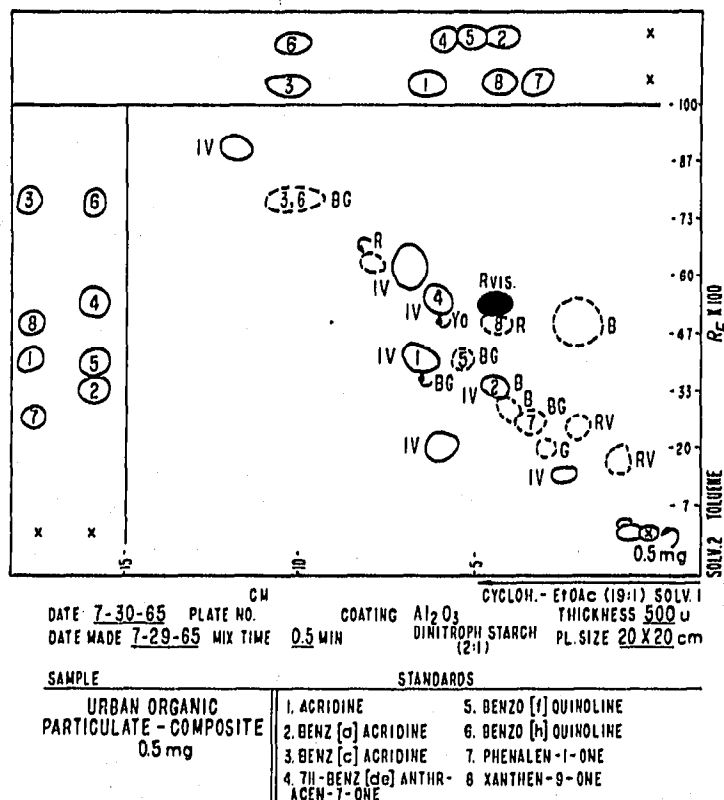


Fig. 3. Two-dimensional thin-layer chromatogram on alumina-2,4-dinitrophenoxy starch (2:1) of the composite airborne particulate sample. Definitions as in Fig. 2; in addition R = red, V = violet.

Separation of the same mixture on alumina-dinitrophenoxy starch (2:1), Fig. 3, gave fewer fluorescent spots, of which approximately half were readily characterized. Apparently the fluorescent spots containing polynuclear aromatic hydrocarbons and some polynuclear heterocyclic compounds were quenched. Treatment of this plate with trifluoroacetic fumes brought out the fluorescence of many of the polynuclear aza and carbonyl compounds.

Although the quencher used in this procedure definitely proved the usefulness of this technique, it presented one serious drawback. When treated with trifluoroacetic acid fumes, the plate acquired a blue fluorescent background color. This fluorescence interfered with direct fluorometric examination of the plate. We overcame this difficulty by eluting the appropriate spot, placing the solution of the spot on glass-fiber paper, treating with trifluoroacetic acid fumes, and examining the area fluorometrically. In this fashion spots containing acridine, benz(*a*)acridine, benz(*c*)acridine, 7H-benz(*d,e*)anthracen-7-one, benzo(*f*)quinoline, benzo(*h*)-quinoline,

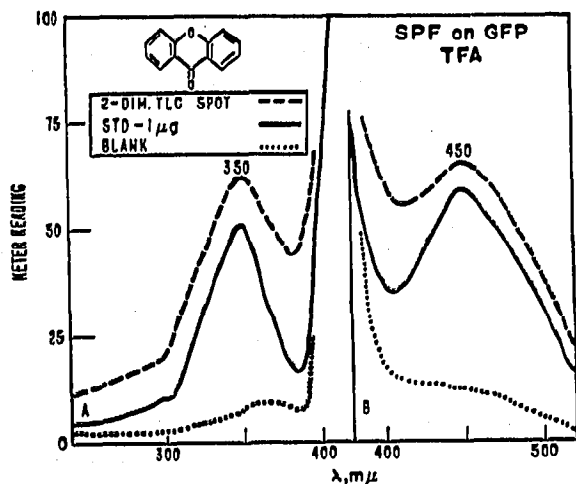


Fig. 4. Fluorescence excitation (A) and emission (B) spectra on glass-fiber paper treated with trifluoroacetic acid of a two-dimensional alumina thin-layer chromatographic spot (see Fig. 3, No. 8) (---), 1  $\mu$ g of xanthen-9-one (—), and the blank (···).

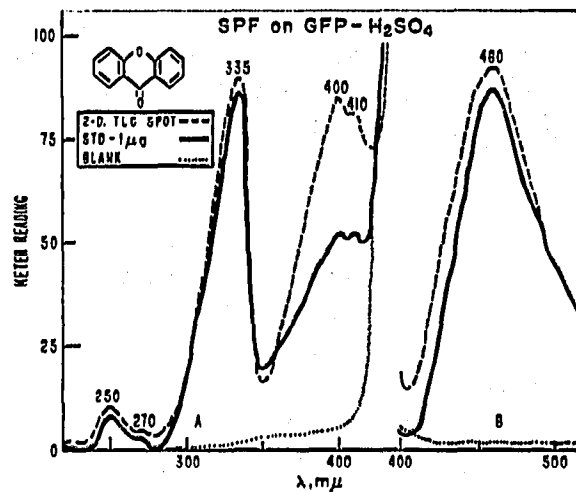


Fig. 5. Fluorescence excitation (A) and emission (B) spectra on glass-fiber paper treated with sulfuric acid of spot no. 8 in Fig. 2: Unknown (---) and standard xanthen-9-one (—). Blank (···).

phenalen-1-one, and xanthen-9-one were readily characterized (Fig. 3). Examples of the characterization are given in Figs. 4 and 5. The fluorescence spectra of the spot were closely similar to that of xanthen-9-one whether the spot was treated with trifluoroacetic acid (Fig. 4) or sulfuric acid (Fig. 5). Xanthone has never been found in the air previously.

An aromatic fraction obtained from an organic airborne particulate sample from Detroit was separated on alumina two-dimensionally with pentane followed by cyclohexane. More than 50 spots were found on the plate. When this same fraction was separated on an alumina-dinitrophenoxy starch (2:1) plate in the same way, only eight spots were obtained. Treatment with trifluoroacetic acid fumes did not bring out any other fluorescent spots. This result indicates the absence of weakly basic polynuclear ring-carbonyl and the somewhat more basic aza heterocyclic compounds.

The two-dimensional separation on alumina of a coal-tar-pitch basic fraction with cyclohexane-ethyl acetate (9:1) followed by toluene showed the presence of more than 50 fluorescent spots. The basic fraction is extremely complicated and contains thousands of compounds<sup>11</sup>. When the same sample was separated on alumina-dinitrophenoxy starch plate, only eight fluorescent spots could be seen. Treatment with trifluoroacetic acid brought out about eight more spots, five of which contained the aza compounds characterized in Fig. 3. Obviously, the use of an appropriate polymeric quencher in the adsorbent on a thin-layer plate should facilitate the characterization and estimation of fluorescent compounds. Many such polymers should be prepared and tested for such applications.

The effect of various types of quenching agents in the adsorbent is shown in Table I. These quenchers could not be used with any developer that would dissolve them. At most they could be slightly soluble in the developer, or else they could be incorporated into an insoluble polymer before use. Their selective quenching efficiency depends to a large extent on the hydroxy, amino, hydrazino, and nitro functional

groups. Thus, morin would be the only compound of the six to fluoresce when the adsorbent contains 1-nitropyrene, and 8-aminofluoranthene would fluoresce in the presence of aniline, *o*-cresol, phenylhydrazine or phloroglucinol while benz(*c*)acridine would not. Table I indicates other possibilities.

#### SUMMARY

Eight fluorescence quenching techniques of value in the direct analysis of spots on thin-layer chromatograms are discussed.

Use of an insoluble quencher in the adsorbent is of benefit in the selective analysis of fluorescent compounds. It is predicted that with the help of polymeric materials containing nitro, phenolic, amino, anilino, thiocarbonyl, ketonic carbonyl, hydrazine, azo, and nitroso groups quick highly selective methods of direct quenchofluorometric analysis of spots on a plate will be possible for compounds containing various types of functional groups. This type of functional group analysis should approach the simplicity of colorimetry.

Examples are given of the application of some of these techniques to the analysis of urban air pollutants. The following compounds have been readily and quickly characterized by the fluorescence quenching techniques: acridine, benz(*a*)acridine, benz(*c*)acridine, 7H-benz(*d,e*)anthracen-7-one, benzo(*f*)quinoline, benzo(*h*)quinoline, phenalen-1-one and xanthen-9-one. All of these compounds have been found in the examined polluted urban atmospheres.

#### REFERENCES

- 1 J. W. SEASE, *J. Am. Chem. Soc.*, 69 (1947) 2242 and 70 (1948) 3630.
- 2 J. G. KIRCHNER, J. M. MILLER AND G. J. KELLER, *Anal. Chem.*, 23 (1951) 420.
- 3 E. STAHL, *Chemiker Ztg.*, 82 (1958) 323.
- 4 E. SAWICKI, W. C. ELBERT AND T. W. STANLEY, *J. Chromatog.*, 17 (1965) 120.
- 5 N. SEILER AND M. WIECHMANN, *Z. Physiol. Chem.*, 337 (1964) 229.
- 6 T. W. STANLEY AND E. SAWICKI, *Anal. Chem.*, 37 (1965) 938.
- 7 E. STAHL, *Thin-Layer Chromatography*, Academic Press, New York, 1965.
- 8 J. M. BOBBITT, *Thin-Layer Chromatography*, Reinhold, New York, 1963, p. 87.
- 9 Z. STRANSKY, *Chem. Listy*, 55 (1961) 1084.
- 10 E. SAWICKI, T. W. STANLEY AND W. C. ELBERT, *Talanta*, 11 (1964) 1433.
- 11 E. SAWICKI, T. W. STANLEY AND W. C. ELBERT, *Occupational Health Rev.*, 16 (1964) 8.

*J. Chromatog.*, 23 (1966) 142-148