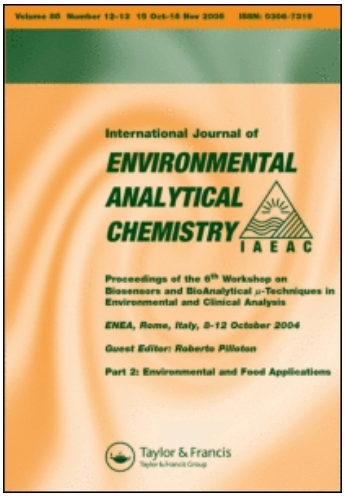

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Fluorimetric Estimation of Aliphatic Hydrocarbons in Airborne Particulates†

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A thin-layer chromatographic method is presented that allows determination of long-chain aliphatic hydrocarbons in the benzene-soluble fraction of airborne particulate extracts by direct scanning in a spectrophotofluorimeter. Silica-gel plates were impregnated with rhodamine 6G, and after development the separated aliphatic hydrocarbon spot was scanned on a scanning spectrophotofluorimeter and measured. The area under the peak was concentration-dependent, with a useful range of analysis from 0.1 mcg to at least 10 mcg of aliphatics in terms of *n*-docosane. Urban air samples contained 1.6 to 2.7 mcg of aliphatics per cubic meter; nonurban samples had 0.12 to 0.24 mcg. Since hydrocarbon carcinogens are ubiquitous, it is suggested that their concentration is of secondary importance in many cases, whereas the much higher concentrations of the cocarcinogens (such as large aliphatic hydrocarbons) and prolonged exposure to them are probably of prime importance.

A number of polynuclear aromatic hydrocarbons and pesticides were screened and were found not to interfere with the determination of the aliphatic hydrocarbons. Their chromatographic and fluorimetric properties under experimental conditions are discussed.

INTRODUCTION

The presence of long-chain normal alkanes in the range of $C_{18}H_{38}$ to $C_{34}H_{70}$ in polluted air and tobacco smoke is well documented.[1–5] Although noncarcinogenic by themselves, these ubiquitous compounds may act as cocarcinogens and cancer-enhancing agents.[6]

Horton *et al.* reported that in mice the production of skin tumors caused by polynuclear hydrocarbons such as benzo(a)pyrene (BaP) or 3-methylchol-

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anthrene was accelerated when the carcinogens were applied topically in the presence of normal alkanes.[7] More recently, Bingham and Falk demonstrated a thousandfold increase in the carcinogenicity of BaP and benz(a)anthracene when *n*-dodecane was used as diluent.[8] The effect of the cocarcinogens was strongest when the concentration of the applied polynuclear hydrocarbons was kept low.

The implications of these findings are obvious. Carcinogens are usually present in the air in minute amounts together with many chemical compounds including long-chain saturated hydrocarbons. Man is continuously exposed to these pollutants in urban air, cigarette smoke, combustion products, and in degradation, oxidation, and volatile products from coal tar and petroleum. It is therefore easy to see that a seemingly nonhazardous amount of polynuclear hydrocarbon emissions can become hazardous if long-chain aliphatics are present in the atmosphere. Few of these alkanes have been studied for their cocarcinogenic activity, but considering their structural similarity, the assumption that many of them possess cancer-promoting properties is reasonable. We decided, therefore, that it was more important to examine the class of long-chain aliphatic hydrocarbons as a whole than to look for individual members. The available methods consist of time-consuming, costly, and not very sensitive procedures. They involve clean-up procedures of the benzene-soluble fractions of air particulate extracts by alumina column chromatography, bromination to eliminate alkenes, extraction of the alkanes, alumina chromatography again, followed by molecular-sieve adsorption chromatography, and, finally, gas chromatographic analysis.[3] The need for a quick, reliable and economical method led to the development of the TLC method reported here. The benzene-soluble fractions of airborne particulate extracts were separated on silica-gel TLC plates impregnated with rhodamine 6G along with standard solutions of *n*-docosane. The aliphatic spots were then scanned on a spectrophotofluorimeter measuring with the two monochromators set at a longer wavelength than the emission wavelength of the dye. Fluorescence and fluorescence quenching at or near the fluorescence excitation and emission wavelength maxima of the dye in the alkane spot can also be used but are less sensitive.

EXPERIMENTAL

Reagents

Cyclohexane† (certified A.C.S. spectranalyzed) obtained from the Fisher Scientific Company (Pittsburgh, Pa.) was redistilled only if it was found to

†Mention of a specific product or company name does not constitute endorsement by the Environmental Protection Agency.

contain interfering contamination substances. All solvents were checked for purity by TLC on silica-gel plates. Long-chain aliphatic hydrocarbons (99% pure) were obtained from Analabs (North Haven, Conn.) and Applied Sciences Laboratories (State College, Pa.). Polynuclear aromatic hydrocarbons and pesticides were obtained from various commercial sources in the purest possible form and were used without further purification. Rhodamine 6G, National Biological Stain, Color Index 45160, was obtained from Allied Chemical (Morristown, N.J.). Silica-gel plates were obtained from the Brinkmann Company (Westbury, N.Y.).

Air particulate samples

Benzene-soluble fractions extracted routinely from airborne particulate material by the method of Tabor *et al.*[9] were obtained from the National Air Surveillance Network of the Environmental Protection Agency. Aliphatic fractions isolated from benzene-soluble fractions by column chromatography were also analyzed.[9]

Equipment

Intensity measurements were made *in situ* by reflectance, according to the Perkin-Elmer manual, on a Hitachi Perkin-Elmer MPF-2A spectrophotofluorimeter equipped with a TLC scanner and were plotted on a strip chart recorder with a chart speed of 50 mm/min. Preliminary studies were performed with a Farrand UV-VIS Chromatogram Analyzer.

Infrared spectra were recorded on a Perkin-Elmer 621 infrared grating spectrophotometer using a rocksalt plate and microfilm technique with beam condensor.

PREPARATION OF TLC PLATES AND CHROMATOGRAPHIC PROCEDURES

An adequate volume of stock solution containing 200 mg rhodamine 6G per 100 ml methanol was diluted 250-fold with methanol. A TLC developing tank was filled with this working solution so that 20×20 cm TLC plates were fully immersed except for the top edge which was left exposed to allow handling of the plates. The plates were left overnight (16 hr) in the dye solution in a tank tightly sealed with Teflon tape instead of a lubricant. Only silica-gel plates containing a binder could be used; otherwise the silica-gel layers would float off in the dye bath. The tanks holding the light-sensitive dye solution, impregnated TLC plates, and development chambers were all protected from light. Four to eight 20×20 cm TLC plates were impreg-

nated simultaneously, air-dried in a horizontal position, fully dried at 40°C under reduced pressure, and stored in closed containers to minimize atmospheric contamination. The impregnated plates were checked under u.v. light, and only those that showed uniform fluorescence were used for quantitative work. Immediately before use, the plates were predeveloped in cyclohexane to the top of the plate.

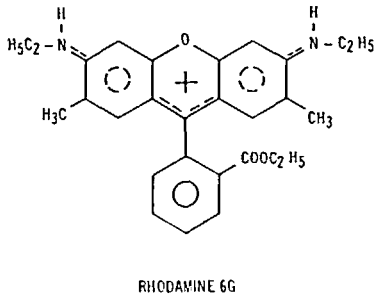
In general, impregnated plates, when properly stored, kept for several weeks without fading. Faded plates were restored by renewed impregnation. The dye bath remained usable for more than a month and could be restored by adding a small amount of the rhodamine 6G stock solution to the tank.

Serial dilutions of *n*-docosane were prepared from a stock solution containing 20 mg of *n*-docosane per ml of chloroform and 1-mcl aliquots were spotted on the TLC plate in single applications, 2½ cm apart. Micropipets used for spotting were checked for uniform flow rates. This, together with a uniform spotting technique, was essential in obtaining reproducible results. The plates were developed in cyclohexane under saturated tank conditions for 35 to 40 min until the solvent front was 10 cm above the origin. The alkanes, which showed as pink spots on the air-dried salmon-colored plate, had an R_f value of approximately 0.67. When chloroform-ethyl acetate (1 : 1) was used as developer, the R_f value was slightly lower. Under u.v. light the spots fluoresced yellow-orange against a lime-colored fluorescent background. The striking change in fluorescence at the spot is derived from the solvent effect of the alkane on the fluorescence emission of rhodamine 6G.

The change in the intensity reading of an alkane spot versus background is best recorded at F 560/592 even though the best fluorescence scan is about F 545/554 (the dye fluoresces maximally at F 525/554 in an alkane environment on the plate) and the best fluorescence quenching scan is at about F 520/544 (the dye fluoresces maximally at F 520/548 on the clean plate). Essentially the fluorescence of the dye in the alkane spot is quenched at or near F 520/548 relative to the fluorescence of the background area at these same wavelengths. Essentially the procedure involves excitation at 560 nm and reading emission intensity at 592 nm. The peak area of the recorded scan is then measured either by planimetry or by triangulation where the area, A, equals the width at 1/2 peak height times peak height.

RESULTS AND DISCUSSION

The structural formula of the xanthene derivative, rhodamine 6G, also known as calcozine red 6G-extra, rhodamine 6GX, or rhodamine 6GDN-extra, is shown in Figure 1. Generally the dye is used as a visualization spray reagent, but a more uniform distribution, and consequently a smoother baseline in the



RHODAMINE 6G

FIGURE 1 Structural formula of the xanthene dye, rhodamine 6G.

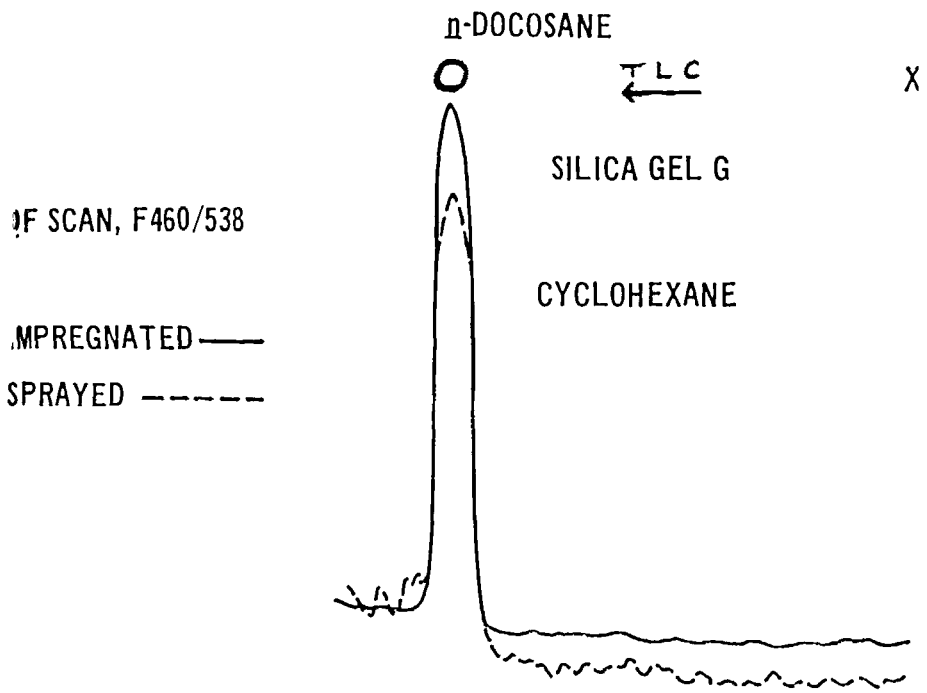


FIGURE 2 Comparison of background noise in scans of docosane *in situ* of a TLC plate impregnated with rhodamine 6G and one sprayed with the same dye after completed chromatography. Developer, cyclohexane; time of development, 40 min; sensitivity, 1; excitation slit, 6 mμ; emission slit, 8 mμ; scan slit, 1 mm; scan speed, 50 mm/min; chart speed, 50 mm/min.

recorded scan, is obtained from an impregnated TLC plate as shown in Figure 2.

The fine structure observed in the excitation spectra is an artifact caused by the absorption bands from the xenon lamp used as the source of light energy.

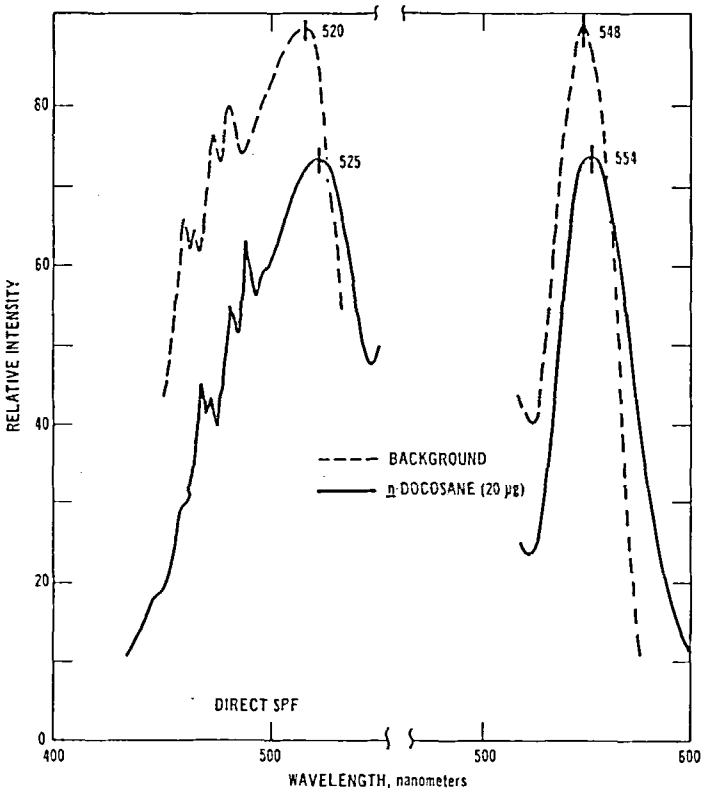


FIGURE 3 Excitation and emission spectrum of a silica-gel TLC plate impregnated with rhodamine 6G and superimposed on it spectra showing the shift in wavelength maxima caused by *n*-docosane. Fine structures at the left side of the excitation spectra are artifacts caused by the absorption bands of Xenon light energy source.

Figure 3 shows excitation and fluorescence spectra of the blank impregnated plate, and superimposed on them is the shift in wavelength maxima caused by the alkane spot. The quenching phenomenon and the change in fluorescence color of the alkane spot are derived from the environmental effect on the absorption and emission spectra of rhodamine 6G. This dye has a cationic resonance structure. The long wavelength absorption band derived

from this type of structure shifts to longer wavelengths when its environment is changed from the polar silica-gel type to the nonpolar alkane type. As the energy of the ground state of this symmetrical molecule is lowered, so is the energy of the excited state. Thus the emission also shifts to longer wavelengths. If the spot is examined at the maximum excitation wavelength of the background dye, quenching is observed. But if the spot is examined at somewhat longer excitation wavelengths (λ532 to λ545) than that at which the background

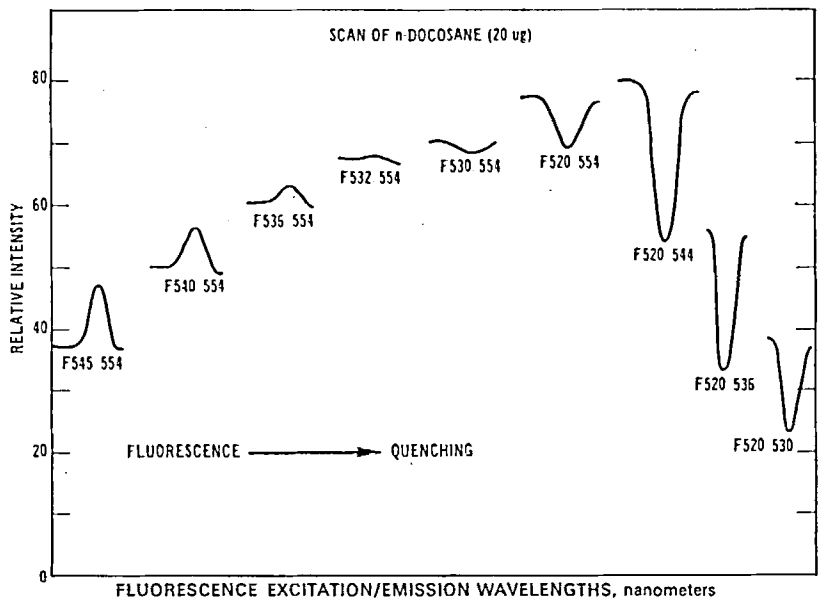


FIGURE 4 *In situ* TLC scans of *n*-docosane on a rhodamine-6G-impregnated silica-gel plate measured at different wavelength settings to illustrate the conversion of fluorescence into quenching. Baseline not adjusted. Change in baseline is due to background fluorescence, of rhodamine-6G-impregnated silica-gel plate.

dye absorbs, the fluorescence of the dye in the nonpolar environment is obtained (Figure 4).

Although maximal fluorescence intensity of the dye in the alkane spot was obtained at F 525/554, the spots were scanned at F 560/592, since at these wavelengths the largest signal was obtained.

Alternatively, at F 470/530 fluorescence quenching could be used to estimate the concentration of alkane in the alkane spots.

The influence of background fluorescence on the scan of the sample is illustrated in Figure 4. In this run, a single docosane spot was scanned at various wavelengths without making baseline adjustments. Each baseline, therefore, represents the fluorescence intensity of the rhodamine-6G-impreg-

nated plate, and the sample peak is the difference between the intensity of the sample and that of the plate at that particular wavelength. As the fluorescence of the background increased, the sample peak decreased, even though the actual recorder reading increased. At F 532/554, the sample peak is indistinguishable from the baseline, although the intensity is about 70% of the recorder scale. In the plot at F 520/554, the fluorescence of the plate is still increasing while the docosane spot begins to act as fluorescence quencher.

As in any other TLC method, reference standards must be included in each sample run. Slight differences in color tinges may occur between different batches of TLC plates, between different batches of rhodamine dye preparations, or between plates impregnated on different days. Fluorescence spectra of impregnated plates from various batches showed differences of 2 to 3 nm in wavelength maxima. For any single run however, the method was reproducible. Sixteen *n*-alkanes ranging from $C_{10}H_{22}$ to $C_{36}H_{74}$ were analyzed. Dotriacontane, tetratriacontane, and hexatriacontane all had an R_f value of 0.69, whereas alkanes with less than 33 carbon atoms consistently showed a slightly lower R_f value. Hexadecane and alkanes with lower carbon content were unstable: the color and fluorescence of the spot faded within a short time after development. Because of their volatility they have never been collected in airborne particulate extracts. All the alkanes examined on rhodamine-6G-coated silica-gel plates showed the same wavelength maxima, and *n*-docosane was chosen as the standard primarily because it was the least expensive. The fluorescence (derived from the dye) of the alkane spots was stable for at least 16 hr provided the thin-layer chromatograms were protected from light; in fact, we obtained identical intensity readings for as long as 48 hr after the initial scan. The peak area in scans of *n*-docosane and other normal alkanes was concentration-dependent. Several concentration ranges were analyzed. Preparation of two standard curves was necessary because the lower concentrations required a higher sensitivity setting on the recorder. The reproducibility of the method is shown in Figure 5, which represents scans in the range of 0.125 to 2.0 mcg. The straight-line relationship obtained is shown in Figure 6. At the 1-mcg level, with $n = 14$, the relative standard deviation was 5.4%. Since the intensity of the spot was measured against a fluorescent background by using a recorder of limited zero-suppression capability, a compromise had to be made in adjusting the baseline. When the monochromator slits were opened to increase the sensitivity, the plate fluoresced strongly and the intensity of the spot was off scale. On the other hand, when the sensitivity was reduced to suppress the fluorescence of the plate, the intensity of the sample spot was likewise reduced. Therefore, in order to obtain maximal sensitivity, the intensity of the most concentrated spot was set at or near 100% reading, and the resulting baseline was used for all scans of that particular TLC run.

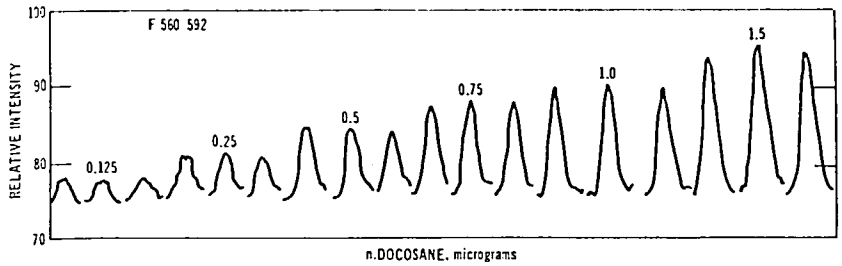


FIGURE 5 Chromatogram of several concentrations of *n*-docosane spotted in triplicate in 1-mcl aliquots on rhodamine-6G-impregnated silica-gel plates and developed for 40 min in cyclohexane. Area under the peak is concentration-dependent.

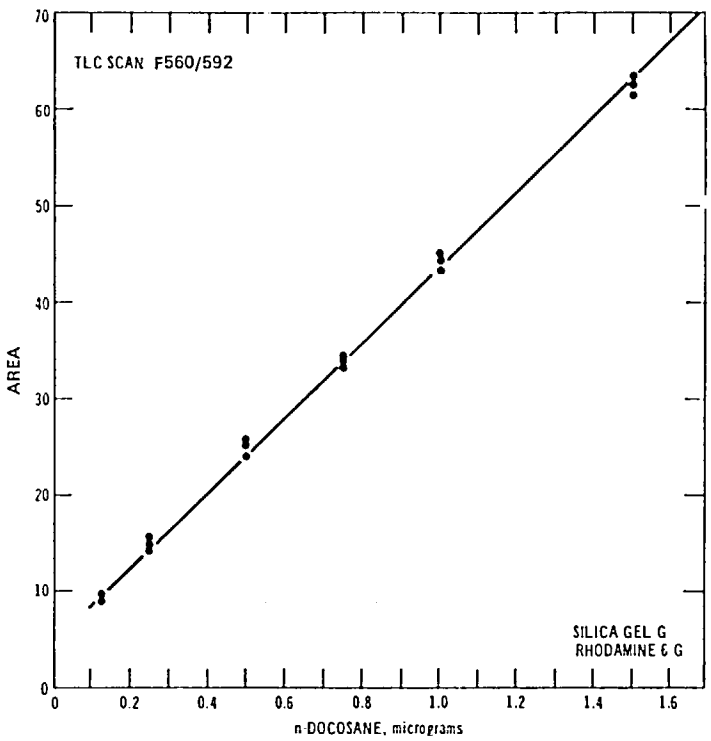


FIGURE 6 Peak areas of *in situ* TLC scans plotted against concentration of standard solutions of *n*-docosane spotted in triplicate on rhodamine-6G-impregnated silica-gel plates. Developer, cyclohexane; developing time, 40 min.

The scans in Figure 7 show the separation of pure BaP and docosane. BaP, an important airborne carcinogen, is used as an index of air pollution. As with most of the polynuclear hydrocarbons tested, BaP gave a negative band at any excitation wavelength, whereas the alkane gave a positive peak at F 560/592 and quenched at F 470/530. The apparent difference in the area of the two BaP peaks results from the difference in baseline setting of the respective scans.

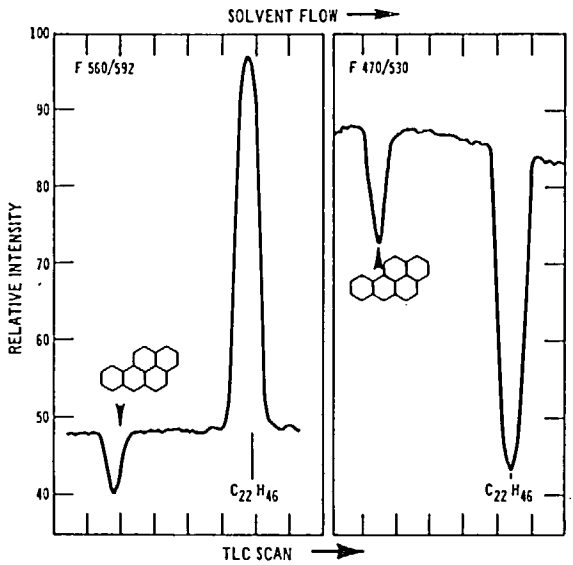


FIGURE 7 Chromatographic separation of a mixture of pure BaP and *n*-docosane on a silica-gel plate impregnated with rhodamine 6G developed for 40 min in cyclohexane and scanned in the direction of the solvent flow. At F 470/530 aliphatics quench the fluorescence; at F 560/592 they show a strong positive response. BaP gives a negative band at any wavelength setting.

The investigated polynuclear aromatic hydrocarbons could easily be differentiated from the aliphatic compounds. Their R_f values were considerably lower and most of them quenched the fluorescence of rhodamine 6G (Table I). Exceptions were fluorene and fluoranthene, which fluoresced brightly on the impregnated plate. This phenomenon is similar to the quenching effect of nitromethane on the fluorescence of large arenes; all are quenched except the fluoranthenes.[10] On rhodamine-6G-coated silica-gel plates, benzene substitution appeared to dull the fluorescence of the fluoranthene: benzo(b)-fluoranthene fluoresced far less than fluoranthene. Benzo(k)fluoranthene showed fluorescence as long as the TLC spot was still moist with the de-

TABLE I

Aliphatic and aromatic compounds analyzed[a]

Compound	100 R_f	Number of rings	Color[b]	U.v. light[c]
Normal alkanes, $C_{33}H_{68}$ to $C_{36}H_{74}$	69.0	0	pk	YF
Normal alkanes, $C_{18}H_{38}$ to $C_{32}H_{66}$	67.0	0	pk	YF
17-Pentatriacontene	67.0	0	pk	YF
Docos-1-ene	65.0	0	pk	YF
Naphthalene	64.5	2	pk	YF[d]
Squalene	62.0	0	pk	YF
Acenaphthene	40.0	3	None	Q
Pyrene	34.3	4	pu	Q
Thioxanthene	34.0	3	None	Q
Anthracene	33.4	3	pu	Q
2-Methylpyrene	31.5	4	pu	Q
2-Methylphenanthrene	31.2	3	pk	YF
Fluorene	31.0	3	pk	YF
Fluoranthene	29.5	4	pk	Pk-W F
Chrysene	25.4	4	pk	Pk F, Center BF
Benzo(a)pyrene	24.0	5	pu	Q
Benzo(ghi)fluoranthene	23.0	5	pk	OF
Benz(a)anthracene	23.0	4	pu	Q
Perylene	22.0	5	y	Q
Benzo(k)fluoranthene	22.0	5	rpu	Q
Azulene	21.7	2	g	Q
Benzo(e)pyrene	21.6	5	pu	Q
Benzo(b)fluoranthene	21.0	5	pk	Pk F
Benzo(ghi)perylene	20.0	6	pu	Q
4H-Cyclopenta(def)phenanthrene	19.8	3	None	Q
Coronene	18.6	7	pu	Q
Dibenzocoronene	14.5	9	pu	Q
9-Nitroanthracene	6.6	3	b	Q
Azobenzene	5.0	2	None	Q
Carbazole	2.5	3	pu	Q
11 H-Benzo(a)carbazole	2.1	4	b-pu	Q
2-Nitrofluorene	2.0	3	pu	Q
1-Nitropyrene	2.0	4	y	Q
Acridine	0.0	3	pu	Q
2-Aminoanthracene	0.0	3	b	Q
7 H-Dibenzo(bg)carbazole	0.0	5	r-b	Q
Pentacene	0.0	5	pu	Q

[a]Developer: Cyclohexane.
Adsorbent: Silica-gel TLC plates impregnated with rhodamine 6G.
[b]b-brown, g-gray, pk-pink, pu-purple, r-reddish, y-yellow.
[c]F-Fluorescence, Q-Quenching, Pk-Pink, O-Orange, W-White, B-Blue, Y-Yellow.
[d]Unstable.

veloper, but, in drying, it appeared as a dull reddish-purple spot under u.v. light, giving a quenching peak on the scan. 2-Methylphenanthrene and chrysene were the other two aromatic hydrocarbons that fluoresced on the

TABLE II

Pesticides giving pink color and yellow fluorescence on rhodamine 6G silica-gel plates[a]

Compound	100 R_f
n-Docosane[b]	64
0(2,4-Dichlorophenyl)0,0-diethylthiophosphate	55
Heptachlor (Minor Q spot, R_f 32)	46
Endrin	43
DDT	42
Ethion	41
(2,4,5-Trichlorophenoxy)acetic acid, isopropyl ester	40
Chlordane[c]	40
2,4-Dichlorophenoxyacetic acid, isopropyl ester	38
Dimethyl-2,3,5,6-tetrachloroterephthalate	37
1,1-Bis(*p*-ethylphenyl)-2,2-dichloroethane	35
4,4'-Dichloro-α-(trichloromethyl)benzhydrol	34 and 53
1-Naphthaleneacetic acid, methyl ester	34
1,1-Bis(*p*-chlorophenyl)2,2-dichloroethane	32
Isopropyl N-(*m*-chlorophenyl)carbamate	28
Tributylphosphorotrithioate	24
Diazinon	19
2,4-Dichlorophenoxyacetic acid, butyl ester[c]	19
Endosulfan	16
Tetradifon[c]	12
Methoxychlor	8
2-Naphthoxyacetic acid (2 minor Q spots R_f 0 and 3)	7
5-Bromo-3-sec-butyl-6-methyluracil[c]	3
Piperonylbutoxide	2
2-Ethyl-1,3-hexanediol	2
2-Ethyl-2-butyl-1,3-propanediol	1
Tributyl(2,4-dichlorobenzyl)phosphonium chloride[c]	1
o-Chlorophenoxyacetic acid	0
1,4,5,6,7,7-Hexachloro-N-(ethylmercuri)-norbornene-2,3-dicarboximide	0
Diphenylacetonitrile	0

[a]Developer: Ligroin-chloroform (1:1).
Developing time: 35 min.
[b]*n*-Docosane is not a pesticide and was used only as a reference marker.
[c]With time, fluorescence changed into quenching.

rhodamine-6G-coated plates. In general, the R_f values of the investigated polynuclear aromatic hydrocarbons could be roughly correlated with the number of rings in the molecules. The more polar heterocyclic compounds,

such as the benzcarbazoles and acridine, as well as nitro- and amino-substituted polynuclear hydrocarbons, and the easily oxidizable pentacene, stayed at or close to the origin. Naphthalene, although similar to the aliphatics in R_f value, color, and appearance under u.v. light did not interfere because it is very volatile and evaporated from the TLC plate within minutes after

TABLE III

Pesticides quenching rhodamine 6G fluorescence[a]

	100 R_f[b]
2-Methylheptyl-4,6-dinitrophenyl crotonate[c]	45 and 15
4,6-Dinitro-2-sec-butylphenol[c]	36 and 4
0-Ethyl 0-*p*-nitrophenylphenylthiophosphonate	32
Heptachlor (also 100 R_f 46, YF)	32
Dicryl	31
Chloranil	25
2,3,4,5-Tetrachlorophenol	23 and 15
4,6-Dinitrocresol[b]	22
Methylparathion	22
Malathion	21, 18, 17
2,4,5-Trichlorophenol	18
2,3-Dichloro-1,4-naphthaquinone	17
Trithion	16 and 5
Parathion	15
2,2′-Methylenebis(4-chlorophenol)	4
Captan	3
Pentachlorophenol	3
1-Naphthaleneacetic acid	3
2-Naphthoxyacetic acid (also 100 R_f 7, YF)	3 and 0
Chloroacetic acid	2
Phenylmercuric acetate	0
Dieldrin	0

[a]Adsorbent: Silica gel impregnated with rhodamine 6G.
Developer: Ligroin-chloroform (1:1).
Developing time: 35 min.
[b]Presence of several spots indicates impurities in pesticide.
[c]Strong yellow color (all other compounds purple color).

development. The few olefins tested were very similar to the normal alkanes. For instance, 17-pentatriacontene had the same R_f value as docosane, whereas the saturated pentatriacontane showed a slightly higher rate of migration that indicates the influence exerted by the one double bond in the long-chain aliphatic hydrocarbon. The presence of olefins, however, could be ascertained by their reactivity with iodine vapor.

A number of pesticides were screened for possible interference with the analysis of aliphatic hydrocarbons. In general, they showed lower R_f values than the aliphatics. They could be divided into three groups dependent on their color as seen by the eye in roomlight and under u.v. light in a dark box: those that showed the same stable visible and fluorescence colors demonstrated by the alkane spots; those that showed these properties initially but that with time changed into dark spots that quenched the fluorescence of rhodamine-6G-coated plates (Table II); and, finally, those that quenched fluorescence from the start (Table III).

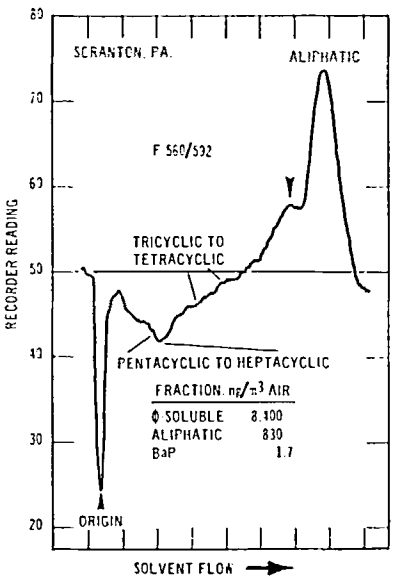


FIGURE 8 *In situ* TLC scan of benzene-soluble fraction from an urban air particulate extract separated on a silica-gel plate impregnated with rhodamine 6G. Amount spotted: 70 mcg of benzene-solubles in 1 mcl chloroform. Developer, cyclohexane; developing time, 40 min. The positive response of the aliphatic hydrocarbons and the negative response of the aromatic hydrocarbons and of the compounds at the origin is evident. TLC spot giving band next to aliphatics (arrow) contains unsaturated aliphatics.

A scan of the benzene-soluble fraction of an urban airborne particulate sample is shown in Figure 8. The more polar constituents stayed at the origin, giving a sharp negative peak. The position of various polynuclear aromatics is indicated on the plot. This was ascertained by spotting individual aromatic compounds alongside the sample, and by adding aliquots of the polynuclears to the sample before spotting. The data on the benzene-soluble fraction placed at the origin are given in this figure.

All airborne samples showed a strong aliphatic peak at the extreme right of the scan. When pure docosane was added to a sample, the height of this particular peak was increased.

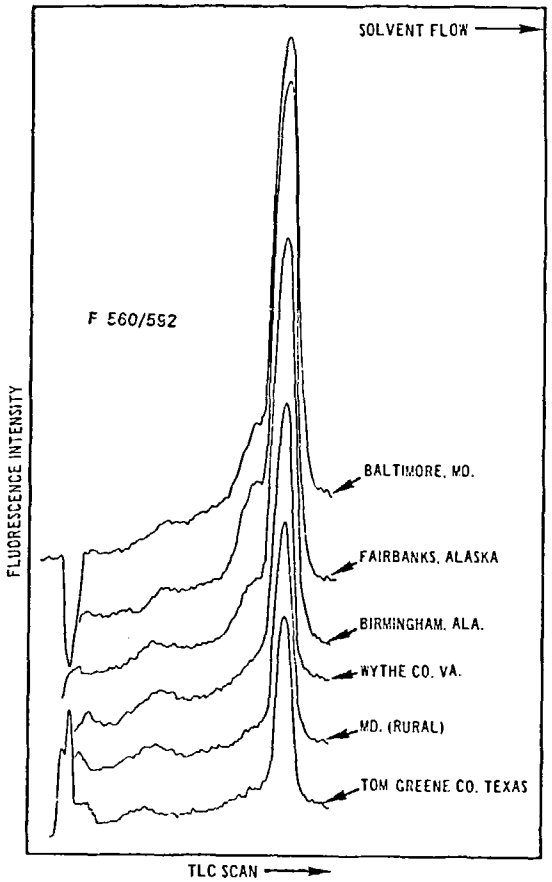


FIGURE 9 *In situ* TLC scans of benzene-soluble extracts from six different collection sites separated on rhodamine 6G silica-gel plates. Tall peaks at right are positive bands derived from the environmental effect of the aliphatic hydrocarbons. Negative band at left is due to quenching compounds remaining at origin. The top five plots showed this negative peak, but four were omitted for reasons of clarity. Bottom scan (T. Greene Co.) was the only one to show positive band instead. All samples adjusted so that each spot contained approx. 30 mcg of benzene-soluble material.

The infrared spectrum of the aliphatic fractions eluted from the plate and purified by repeated TLC showed typical C-H stretching frequencies at 2859 cm^{-1} and 2928 cm^{-1}. Some unsaturation, indicated by a split of the

2928 cm^{-1} band, was probably of an olefinic nature. Methyl and methylene bending vibrations were found at 1378 cm^{-1} and weak C-C stretching frequencies at 729 cm^{-1} and 722 cm^{-1}.

In the u.v. range the eluted material showed a band of low intensity at 250-nm wavelength; this band indicated unsaturation since alkanes do not absorb above 200-nm wavelength.

TABLE IV

Amounts of benzo(a)pyrene and particulate aliphatic hydrocarbons in ambient atmospheres

Sampling site[a]	Air volume (m^3)	BSO[b] mg/air vol.	BSO[b] mcg/m^3	BaP (ng/m_3)	Aliphatic (mcg/m^3)	Ratio, aliphatic to BaP
Fairbanks, Alaska	3475	33.5	9.64	3.18	2.70	849
Birmingham, Ala.	3090	29.9	9.67	8.25	2.60	315
Baltimore, Md.	2484	14.8	5.96	2.47	1.60	648
Calvert Co., Md. (rural)	7972	13.1	1.64	0.64	0.16	250
Tom Green Co., Texas	9656	13.1	1.36	0.17	0.12	706
Wythe Co., Va.	9951	12.3	1.24	0.79	0.24	304

[a] 1st Quarter 1970
[b] BSO = Benzene-soluble organic fraction of airborne particulates.

The presence of some unsaturated material was further confirmed by exposing the plate containing the purified aliphatic spot to iodine vapor. At low concentration, no browning occurred and the homogeneous spot appeared to consist of saturated material only. When the concentration was increased sufficiently, however, two overlapping spots were observed, the lower one reacting with iodine. The amount of olefins present differed for various samples.

The scans of the benzene-soluble filter extracts from three urban and three nonurban air particulate samples are shown in Figure 9. One of the nonurban samples showed a positive peak at the origin instead of the negative peak generally found. The urban samples contained considerably more aliphatics than the nonurban extracts (Table IV). Sample concentration had been adjusted so that each microliter of aliquot spotted contained approx. 30 mcg of the benzene-soluble fraction, which required the collection of an average air volume of 3.5 m^3 for the urban and 20.4 m^3 for the nonurban samples. The ratio of aliphatics to BaP ranges from 315 to 849, varying from sampling site to sampling site. The average value of 439 compares well with approximations obtained earlier.[11]

Contamination of TLC plates and equipment by the ubiquitous aliphatic hydrocarbons may become a problem. Organic solvents, glassware, table tops, and fingertips are subject to this type of contamination. In laboratories where cool or hot air is blown into the room from ducts in the ceiling, we have found considerable air pollution by aliphatics, expecially during a seasonal change-over from heating to air conditioning or vice versa.

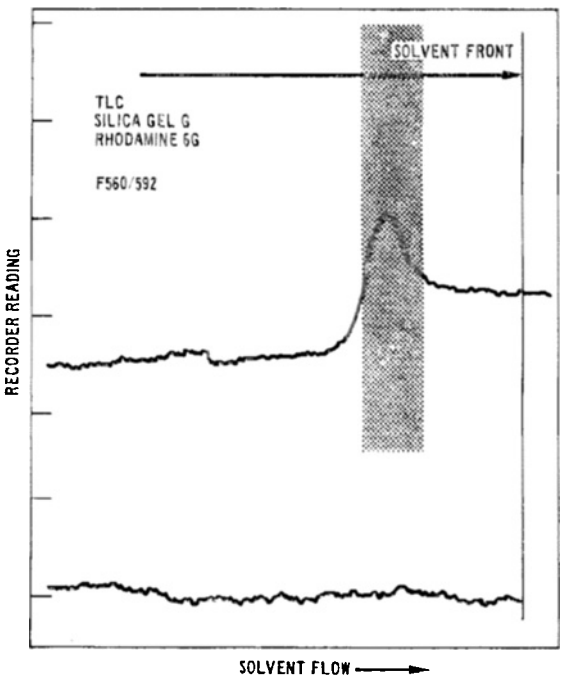


FIGURE 10 Fluorimetric scan of a rhodamine 6G silica-gel plate exposed to laboratory air for 36 hr and developed for 35 min in cyclohexane. The shadowed region represents the pink band observed after development, giving the typical appearance of aliphatics. Higher baseline at right of peak shows portion of plate where hydrocarbon contamination is still present. Flat scan at bottom represents control plate.

Figure 10 shows the scans of two TLC plates from the same batch and predeveloped in cyclohexane; one of which was exposed for 36 hr to the air in a darkened laboratory while the other was protected from the air in a closed container. Both plates were then developed simultaneously and scanned. A broad pink band on the exposed plate gave the typical R_f value and fluorescence spectra found with the aliphatic hydrocarbons. The control plate showed no sign of contamination.

This sensitive method appears well suited to the direct measurement of room air contamination by saturated hydrocarbons.

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