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SEPARATION AND DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND NITROGEN HETEROCYCLES PERTAINING TO COAL GASIFICATION, TAR SANDS, SHALE OIL, COAL LIQUIDS, AND RELATED SAMPLES

Final Technical Progress Report for January 1978—December 1979

By
R. J. Hurtubise
J. D. Phillip
C. D. Ford
R. A. Dalterio

Work Performed Under Contract No. AS20-79LC01761

The University of Wyoming
Chemistry Department
Laramie, Wyoming

U. S. DEPARTMENT OF ENERGY

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Final Technical Progress Report
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Principal Investigator: R. J. Hurtubise
Research Associate: J. D. Phillip
Graduate Students: C. D. Ford and
R. A. Dalterio (one summer)

Chemistry Department
The University of Wyoming
Laramie, Wyoming

Prepared for Laramie Energy Technology Center
Department of Energy
Laramie, Wyoming

LETC Contact Officer: Dr. John McKay

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ABSTRACT

A method was developed for identifying and determing benzo[a]pyrene in a filtered retort water sample from an in situ oil shale process by employing liquid-liquid extraction, dry-column chromatography, thin-layer chromatography and fluorescence spectrometry. The accuracy and precision of the method were good, and the limit of detection was approximately 0.08 ppb. Irreproducible results were obtained with unfiltered retort water samples.

Programmed multiple development was used to a limited extent in the separation of polycyclic aromatic hydrocarbons from a shale oil sample. However, results indicated that this separation technique should be investigated further in the separation of polycyclic aromatic hydrocarbons.

Benzo[a]pyrene was determined in several shale oil samples by methods developed previously. Polycyclic aromatic hydrocarbons were characterized in several shale oil samples using open-column, dry-column and thin-layer chromatography as separation steps. Visible fluorescence from the separated components on chromatoplates was measured directly to obtain fluorescence profiles of the components on the chromatoplates. Several comparisons were made among the shale oil samples as to the relative amounts of 6-, 5-, 4- and 3-ring polycyclic aromatic hydrocarbons.

The theoretical and practical analytical aspects of the room temperature phosphorescence of nitrogen heterocycles were investigated. Intense room temperature phosphorescence was obtained from several nitrogen hetero-

cycles when these compounds were absorbed on silica gel. Theoretical explanations were given for the intense phosphorescence. Benzo[f]quinoline and phenanthridine were separated from a shale oil sample by open-column and high performance liquid chromatography, and then identified by room temperature phosphorescence.

A. RETORT WATER

A method was developed for determining benzo[a]pyrene (BaP) in filtered Omega-9 retort water. The method was also applied to other retort water samples but these samples were not tested as extensively as the Omega-9 sample for reproducibility, percentage recovery, and interferring components. Also, some of the work on Omega-9 retort water was done under previous financial support from DOE-LETC. The fundamental basis for the method was developed by us under that previous support. The basis for the method is described in the following publications:

> R. J. Hurtubise, J. F. Schabron, J. D. Feaster, D. H. Therkildsen, and R. E. Poulson "Fluorescence Characterization and Identification of Polynuclear Aromatic Hydrocarbons in Shale Oil," Anal. Chim. Acta, 89, 377 (1977).
>
> R. J. Hurtubise, G. T. Skar, and R. E. Poulson "Determination of Benzo[a]pyrene in Shale Oil by Solid-Surface Fluorescence," Anal. Chim. Acta, 97, 13 (1978)

The method included three separation steps to isolate BaP, namely, liquid-liquid extraction, dry-column chromatography, and thin-layer chromatography. The quantitation step involved the measurement of the reflected fluorescence of BaP from a thin-layer chromatoplate with a Kontes densitometer. The detailed procedure is given at the end of this section. Full details of the method have been published by us.

> R. J. Hurtubise, J. D. Phillip, and G. T. Skar, "Determination of Benzo[a]pyrene in a Filtered Retort Water Sample by Solid-Surface Fluorescence," Anal. Chim. Acta, 101, 333 (1978).

Table 1 shows recovery data, sample size variation data and BaP content for spiked distilled water samples. For the unfiltered 250-ml samples, good recovery and reproducibility were obtained. With the 500-ml samples, good reproducibility was obtained but the recovery was somewhat low, indicating a sample-size dependency. When the spiked distilled water samples were filtered through Whatman No. 1 filter paper, the percentage recovery was very low (Table 1). Observation of the filter paper under u.v. radiation indicated the bright violet fluorescence of BaP; filter paper has a strong affinity for BaP, and samples should not be filtered through paper before analysis. Filter paper would be very effective for the removal of BaP from water; this is worthy of further investigation.

Table 2 shows recovery data, sample size variation data and BaP content for filtered and unfiltered retort water samples. Several filters (Millipore Corp.) were used in filtering the retort water samples by Laramie Energy

TABLE 1

Percentage recovery and reproducibility of the method for benzo[a]pyrene in distilled water samples

Sample (ml)	BaP added (ppb)	BaP Found (ppb)	Recovery (%)
250	5.0	5.0	100
250	5.0	4.9	98.0
250	10.0	10.4	104
500	5.0	4.4	88.0
500	5.0	4.2	84.0
250[a]	5.0	1.6	32.0
250[a]	5.0	1.0	20.0

[a]Filtered through Whatman No. 1 filter paper.

TABLE 2

Percentage recovery and reproducibility of the method for benzo[a]pyrene in filtered and unfiltered retort water

	Sample (ml)	BaP (ppb) Present	Added	Found	Recovery (%)
Filtered [a]	250	0	5.0	4.5	90.0
		0	5.0	5.1	102
		0	5.0	5.0	100
		0	5.0	5.0	100
		0	10.0	9.40	94.0
		0	10.0	8.64	86.4
	500	0	5.0	3.6	72.0
		0	5.0	3.6	72.0
		0	5.0	4.0	80.0
Unfiltered	250	15.3	10.0	18.8	35.0
		14.4	10.0	24.3	99.0
	500	18.7	-	-	-
		12.4	-	-	-
	250[b]	14.8[c]	10.0	2.6	26.0
			10.0	1.0	10.0

[a]Filtered as described in [1]. [b]Filtered through Whatman No. 1 filter paper. [c]Average of two determinations.

Research Center [1]. BaP was not detected in these samples (Table 2), and 100-µl sample extracts spotted on the chromatoplate showed no BaP. The recovery and reproducibility for BaP in the spiked Millipore filtered samples is good. As with the 500-ml distilled water samples, the 500-ml spiked Millipore filtered samples gave a low percentage recovery indicating a sample size dependency.

The unfiltered retort water samples contained suspended material and were more difficult to handle than the filtered samples. The results in Table 2 for the 250-ml unfiltered retort water samples show a wide variation in percentage recovery. Work with different unfiltered retort water samples

showed a wide variation (Table 2). For two spiked unfiltered retort water samples, filtered through Whatman No. 1 filter paper, the percentage recovery was very low (Table 2). Observation of the filter paper under u.v. radiation showed the characteristic violet fluorescence of BaP.

The poor reproducibility for the unfiltered retort water samples is probably caused by adsorption of BaP on the suspended material in unfiltered retort water; no BaP was found in the filtered retort water samples. When 250-ml samples of unfiltered retort water were filtered through a medium-frit glass filter, BaP was not detected in the filtrate. 1,2-Dichloroethane was passed over the residues in the glass filters, collected, and evaporated to dryness. The residue was dissolved in n-hexane and separated as described under "Experimental" at the end of this section. BaP was detected visually on chromatoplates in both samples. Further work is needed to develop a method for determining the BaP adsorbed on suspended material; the limited number of samples available made it impossible to pursue this. Saxena et al. [2] suggested that part of the increased efficiency of retention of BaP, on porous polyurethane foam, by heating tap water containing BaP, was related to the desorption of BaP from suspended particles in the water. Suspended material in water can have a pronounced effect [3] on the extraction efficiency of PAH from water. It is important to distinguish between dissolved BaP and BaP adsorbed on suspended material. The method developed in this work accurately determines dissolved BaP in water samples that are relatively free of suspended material. The limit of detection for BaP in filtered retort water extract, defined as described previously [4] was 0.9 ppb. When 100 µl of filtered retort water extract was spotted, the background signal was the same as that for 5 µl, and the limit of detection was 0.08 ppb,

which could be decreased even more by spotting more sample on the chromatoplate.

EXPERIMENTAL

Apparatus

The determination of BaP and the recording of fluorescence emission spectra have been described [4].

Materials

The dry column consisted of a 28-cm section of polyethylene tubing (0.25 in. i.d., Curtin Matheson Scientific, Inc.), 23 cm of which was packed with aluminum oxide, activity II to III according to Brockman (ICN Life Sciences Group, Cleveland, Ohio). The bottom of the column was plugged with glass wool. All solvents used were reagent grade. Distilled n-pentane was obtained from Burdick and Jackson Laboratories, Inc., Muskegon, Michigan. Precoated, 20 x 20-cm 30%-acetylated cellulose chromatoplates were used for thin-layer chromatography (t.l.c.) work (Cel 300 AC/30-22 Brinkmann Instruments, Inc., Westbury, New York). The 99+% BaP was obtained from Aldrich Chemical Co., Milwaukee, Wis. A 10-µl Hamilton syringe was used in spotting the chromatoplates. A Selectasol chromatography chamber (Schleicher and Schuell, Inc., Keene, New Hampshire) was employed in determining the eluants to be used in the t.l.c. work.

The retort water samples were obtained from the Laramie Energy Research Center, Department of Energy, Laramie, Wyoming. Unfiltered and filtered (0.4 µm) retort water samples used in development of the analytical method were obtained from the Omega-9 collection (internal designation used by the

Laramie Energy Research Center for process waters obtained during the in situ oil shale retorting experiment at Site 9 near Rock Springs, Wyoming, in 1976). The acquisition, process and storage of the Omega-9 retort water has been described [1]. The Omega-9 retort water collection and the samples used were maintained under refrigerated conditions. Chemical parameters indicative of the composition of the Omega-9 water used are: 3,470±830 mg l^{-1} NH_4^+; 2,740±730 mg l^{-1} $S_2O_3^{2-}$; 15,940 mg l^{-1} HCO_3^-; pH 8.6±0.3; 1,030±105 mg l^{-1} total organic carbon, and 14,510±690 mg l^{-1} total dissolved solids [5].

Procedures

An accurately measured, filtered or unfiltered, retort water or distilled water sample was added to a 1-l separatory funnel. The sample was extracted four times with n-pentane, first with 100 ml for 10 min and then with three 50-ml portions for 5 min each. The n-pentane layers were combined. In the filtered or unfiltered retort water samples, a third layer appeared during the extractions. This middle layer, which was more cloudy with unfiltered samples and appeared to be air and n-pentane trapped within the suspended material of the retort water, was eliminated partially by applying suction to the separatory funnel with a vacuum line. After removal of the n-pentane layer for the last extraction, the middle layer was centrifuged and the resulting n-pentane layer was pipetted into the other n-pentane layers; the combined layers were evaporated to dryness at room temperature in an Erlenmeyer flask. For distilled water samples and filtered retort water samples, the evaporated residue was dissolved in 1 ml of n-hexane and transferred to a 1-ml volumetric flask where the n-hexane

was evaporated to dryness. The Erlenmeyer flask was rinsed with n-hexane and the rinsings were added to the 1-ml volumetric flask. The volume was then diluted to 1 ml with n-hexane. For unfiltered retort water samples, the evaporated residue was dissolved in 1-ml of n-hexane transferred to a 2-ml volumetric flask followed by the Erlenmeyer flask rinsings. The volume was then diluted to 2 ml with n-hexane. A 1 ml volume of n-hexane concentrate from either filtered or unfiltered samples was added to the aluminum oxide column, which was developed with 5 ml of n-hexane-ether (19:1). After development the column was observed with a u.v. handlamp and the purple fluorescent band was sliced with a razor blade from the column to give a 5-cm section. The migration distance to the center of the BaP band was 6.5 cm for BaP in distilled water, 8 cm for BaP in filtered retort water, and 8.5 for BaP in unfiltered retort water. To determine the exact migration distance for BaP from distilled water and retort water samples, a known amount of BaP was added to the samples and the spiked samples were carried through the extraction and column separation steps. The migration distances for the specific samples were very reproducible; after the migration distances had been determined, it was not necessary to run spiked samples. The aluminum oxide from the sliced section was stirred for 30 min with 20 ml of 1,2-dichloroethane. After the solvent had been decanted, the aluminum oxide was washed twice with 10 ml aliquots of 1,2-dichloroethane; the last wash was filtered rather than decanted. The combined extracts were evaporated to dryness under vacuum at room temperature and the residue was dissolved in 1 ml of n-hexane.

This solution (5 µl) was spotted in duplicate on a 30% acetylated cellulose chromatoplate. Standard solutions of BaP in n-hexane at con-

centrations of 2, 6, and 10 ng μl^{-1} were also spotted (5-µl amounts) in duplicate. The plate was developed to 10 cm with methanol-n-hexane-acetone (7:3:2) for distilled water and filtered retort water samples. For unfiltered retort water samples two developments were required because of a fluorescent impurity immediately above BaP on the chromatoplate. The first development was carried out with methanol-water-acetic acid-acetone (20:5:3:3) and the second with methanol-n-hexane-acetone (7:3:2). The chromatoplate was then air-dried and the fluorescence intensity was measured with the Knotes densitometer. Each spot was scanned perpendicular to the direction of development. Recorded peak heights corresponding to fluorescence intensity of the standards were plotted versus nanograms of BaP. The unknown amount of BaP on the chromatoplate was obtained from the calibration curve and calculated as nanograms of BaP per ml of retort water (ppb).

To identify BaP, the fluorescence emission spectra were measured directly from chromatoplates by the procedures described previously [6].

Distilled water and retort water samples were spiked with acetone solutions of BaP. Solutions of BaP in acetone (1 µg ml^{-1} and 2 µg ml^{-1}) were employed to prepare 5-ppb and 10-ppb spiked samples, respectively.

B. PROGRAMMED MULTIPLE DEVELOPMENT (PMD)

PMD is defined as the repeated development of a TLC plate with the same solvent in the same direction for gradually increasing distances. A Regis PMD unit was used in this work. PMD was not used extensively but some useful information was obtained on five ring polycyclic aromatic hydrocarbons (PAH) in some shale oil samples. The five ring PAH were isolated by dry-column chromatography and thin-layer chromatography and the separation procedures were described previously (4). Figure 1 compares the results of conventional TLC with PMD for four PAH standards. As indicated in Figure 1, the additional PMD step gives very good resolution of the four compounds. Figure 2 compares the results with a five ring PAH fraction from a shale oil sample (SOA 70-97). As shown with the additional PMD step, several distinct spots were obtained.

Nitromethane was found by Sawicki, et al. (7) to quench the fluorescence of PAH except those with a fluoranthenic nucleus. This test was applied to some of the components in the five ring PAH fraction from the shale oil sample. Spots 2, 3, and 4 in Figure 2 remained fluorescent while the fluorescence of spots 1 and 5 was quenched. From previous work it was discovered that spot 1 was benzo(a)pyrene. Spot 4 was postulated to be benzo(k)fluoranthene on the basis of fluorescence data. For example, the fluorescence emission spectrum of spot 4, obtained directly from the chromatoplate, matched very well with literature spectra of benzo(k)fluoranthene. Spots 2 and 3 probably are components with the fluoranthenic nucleus because they remained fluorescent in the presence of nitromethane.

Little additional work was done with PMD; however, PMD should be explored further for its potential to separate PAH.

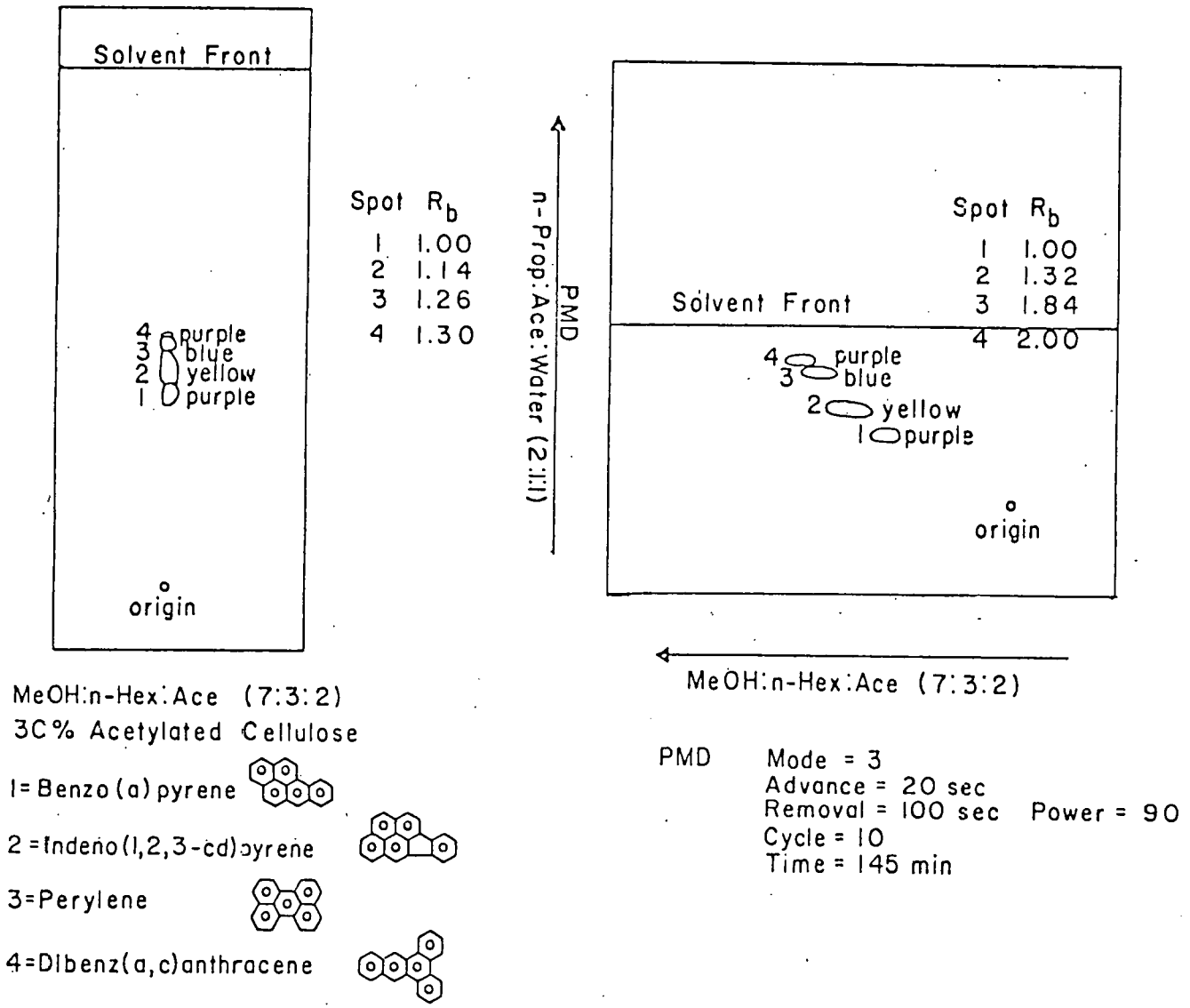
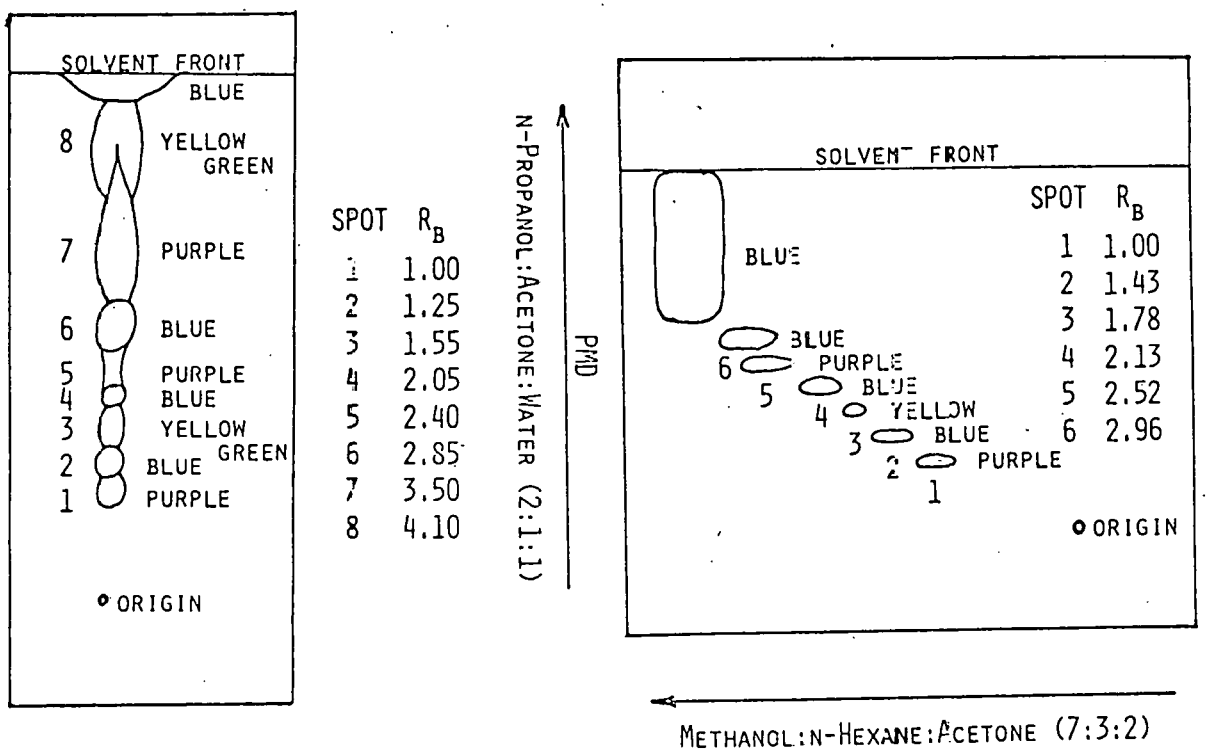

Solvent Front
4 purple
3 blue
2 yellow
1 purple
origin
Spot R_b
1 1.00
2 1.14
3 1.26
4 1.30
MeOH:n-Hex:Ace (7:3:2)
30% Acetylated Cellulose
1=Benzo(a)pyrene
2=Indeno(1,2,3-cd)pyrene
3=Perylene
4=Dibenz(a,c)anthracene
PMD
n-Prop:Ace:Water (2:1:1)
Spot R_b
1 1.00
2 1.32
3 1.84
4 2.00
Solvent Front
4 purple
3 blue
2 yellow
1 purple
origin
MeOH:n-Hex:Ace (7:3:2)
PMD Mode = 3
Advance = 20 sec
Removal = 100 sec Power = 90
Cycle = 10
Time = 145 min

Figure 1

BaP FRACTION SOA 70-97



MOBILE PHASE:

N-PROPANOL:ACETONE:WATER (2:1:1)

STATIONARY PHASE:

30% ACETYLATED CELLULOSE

PMD

MODE = 3
ADVANCE = 20 SEC
REMOVAL = 100 SEC POWER= 9
CYCLES = 10
TIME = 145 MIN

Figure 2

C. DETERMINATION OF BENZO[A]PYRENE AND CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN SHALE OIL SAMPLES, AND REMOVAL OF NITROGEN HETEROCYCLES

Determination of Benzo[a]pyrene in Shale Oil

Benzo[a]pyrene (BaP) was determined in several shale oil samples by methods developed by us (4). The average BaP content of the samples from duplicate determinations is given in Table 3.

TABLE 3

Benzo[a]pyrene Content in Shale Oil Samples

Sample Number Laramie Energy Technology Center	Sample Number University of Wyoming	BaP, ppm
SOA76-173	13	28
SOA77-205	14	52
SOA76-135	15	7.2
SOA77-231	16	11
SOA77-233	17	14
SOA79-1	18	11
SOA79-6	19	11
SOA79-8	20	23
SOA79-10	21	7
SOA79-47	22	346
SOA79-48	23	276

General Characterization of PAH

PAH (6-,5-,4- and 3-ring) were characterized in the first nine shale oil samples listed in Table 3. Open-column, dry-column and thin-layer chromatography were used in the separation steps. Visible fluorescence from the separated components on chromatoplates was measured directly to obtain fluorescence profiles of the components distributed on the chromatoplates (4,6,8). Because of the extensive data obtained in the characterization of the nine shale oil samples, only a small amount of data will be given in this report. Experimental procedures are given at the end of this section.

Snyder [9] has shown that PAH can be separated according to ring size by using an aluminum oxide stationary phase and a relatively weak mobile phase. These concepts were applied in developing separation methods for PAH in shale oil samples [6]. That work has been expanded further, as described here. In the initial dry-column chromatography, aluminum oxide (activity II-III) was the stationary phase and n-hexane-ether (19:1) was the mobile phase. Data from several PAH standards with different ring sizes again indicated that PAH would be separated by ring size with limited overlap of bands. After the particular ring band had been sliced out, the second separation step with t.l.c. was used. The 30% acetylated cellulose was employed in the t.l.c. step because it is useful in separating PAH ring isomer [10], and many PAH give intense fluorescence signals on 30% acetylated cellulose [4,6]. The mobile phase used gave smaller spots, better separation of shale oil components, and distributed the components over a greater distance on the chromatoplate than the mobile phase reported previously [4]. The final characterization step involved measurement of reflected fluorescence from the chromatoplates; thus a fluorescence profile of the separated components was obtained.

Different sample sizes for the dry-column step and different amounts spotted on the chromatoplates for the TLC step were tested so that maximum response and reproducible data could be obtained. Five shale oil samples were run through the entire procedure at least twice. The R_f values corresponding to the major fluorescence peaks in the scans of the chromatoplates were reproducible (±0.02) and the overall shapes of the fluorescence profiles of components separated on chromatoplates were reproducible. The relative fluorescence intensity values for the major peaks in the profiles could be

used as a semi-quantitative measure of various components. It is not surprising that relative intensity values were not highly reproducible; the large number of components in each ring fraction would increase the probability of quenching interactions and of components migrating at somewhat variable rates.

Figures 3 and 4 compare the fluorescence profiles of the 4-ring PAH fractions from samples SOA76-173 and SOA77-205, respectively. The general shapes of the fluorescence profiles for the 4-ring PAH fractions are similar, suggesting a similar composition for the two fractions. However, the relative intensity (peak height) for the 4-ring fraction from sample SOA76-173 is greater than that for sample SOA77-205 indicating a higher concentration of 4-ring PAH in sample SOA-173. Analogous reasoning can be applied to the 3-ring fractions and it appears that there is a higher concentration of 3-ring PAH in sample 13 than in sample 14. In Figures 5 and 6, the fluorescence band at R_f values of about 0.95 was due to fluorescent impurities in the chromatographic system.

The R_f values determined at the fluorescence maxima for the major fluorescence peaks and the relative fluorescence intensity values for the major peaks obtained from the fluorescence profiles for samples SOA76-173 and SOA77-205 are compared in Table 4. For all the ring fractions, sample SOA76-173 yielded greater relative fluorescence intensity values than sample SOA77-205. This indicated that sample SOA76-173 contained a higher concentration of 6-, 5-, 4- and 3-ring PAH than sample SOA77-205. Comparison of R_f values are similar except for the 6-ring fraction. Also except for the 6-ring fraction, each given ring fraction contains the same number of R_f values. These results suggest a general similarity of the composition

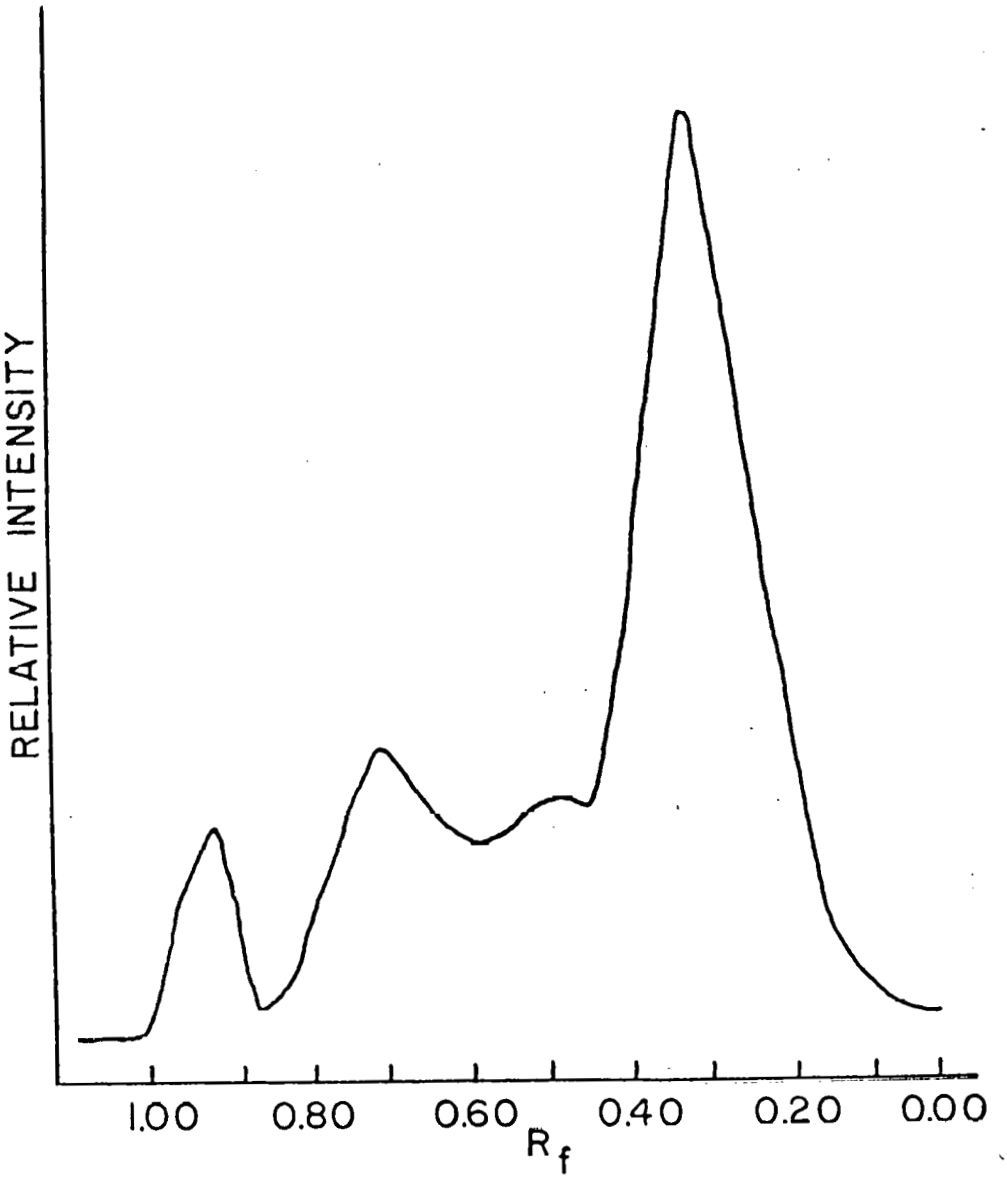


Figure 3. Fluorescence profile of the 4-ring fraction from sample SOA76-173.

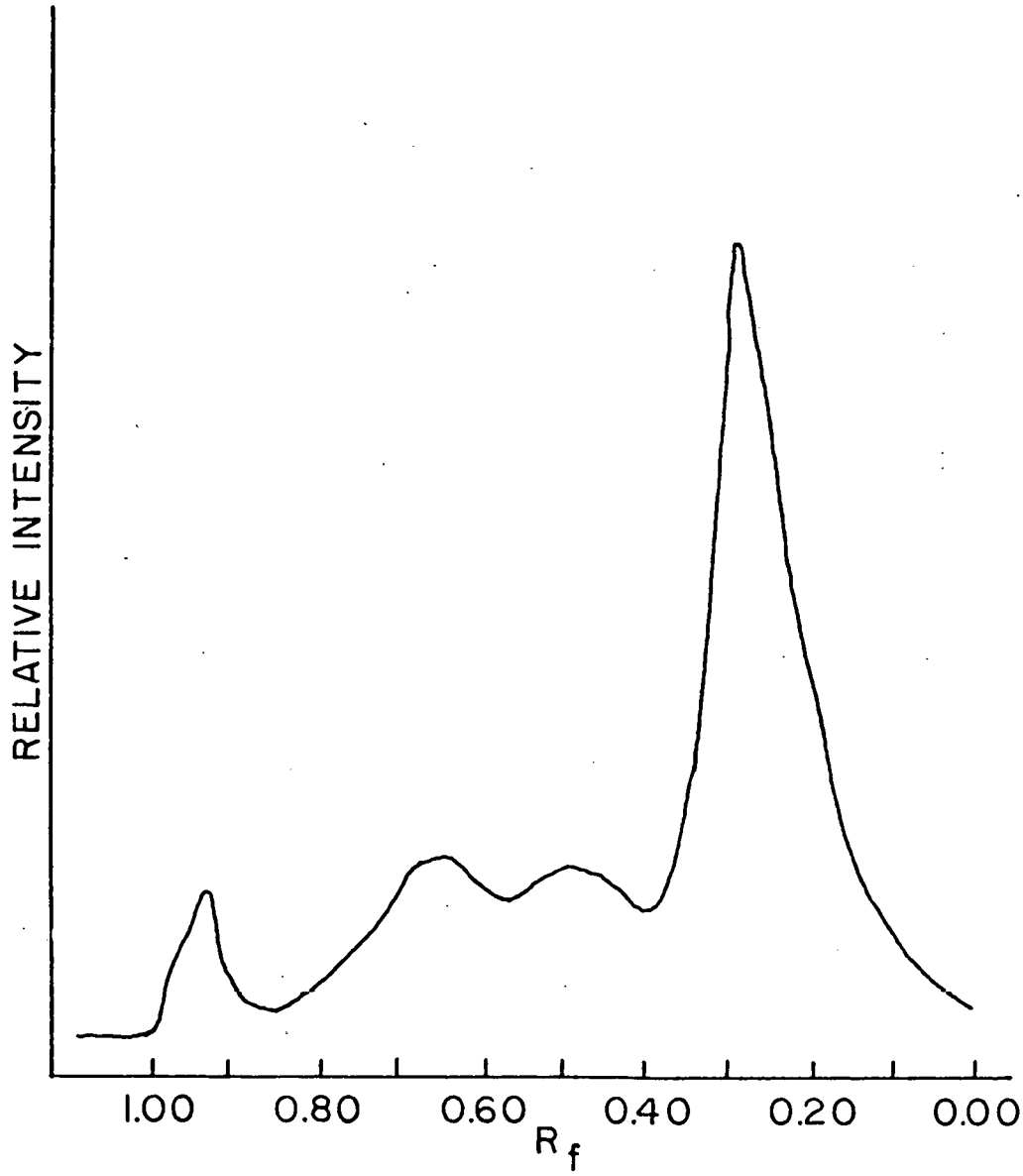


Figure 4. Fluorescence profile of the 4-ring fraction from sample SOA77-205.

Figure 5. Fluorescence profile of the 3-ring fraction from sample SOA76-173

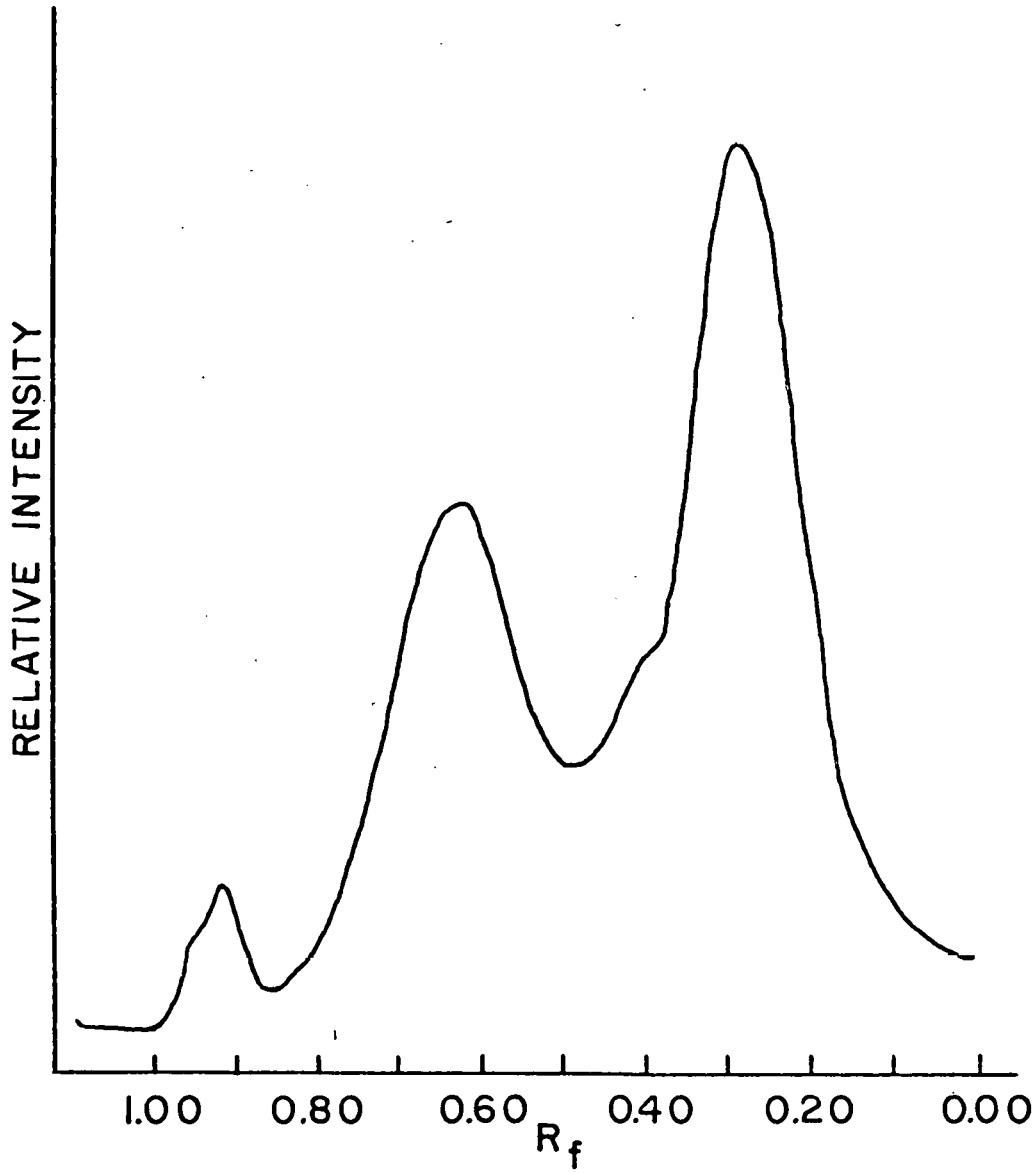


Figure 6. Fluorescence profile of the 3-ring fraction from sample SOA77-205.

TABLE 4

Comparison of R_f and relative fluorescence intensity values

Sample	Ring fraction	R_f	Relative intensity	Sample	Ring fraction	R_f	Relative intensity
SOA76-173	6	0.21	128	SOA76-173	4	0.31	125
		0.96	26			0.51	36
						0.69	48
SOA77-205	6	0.13	97			0.94	42
		0.31	64				
		0.47	47	SOA77-205	4	0.25	60
		0.64	19			0.49	14
		0.92	25			0.64	18
						0.94	15
SOA76-173	5	0.26	120				
		0.51	30	SOA76-173	3	0.35	107
		0.70	21			0.67	61
		0.93	27			0.94	24
SOA77-205	5	0.19	34	SOA77-205	3	0.29	62
		0.48	12			0.61	39
		0.65	12			0.92	16
		0.95	14				

of the various ring fractions. For the 6-ring fractions in Table 4 sample SOA77-205 gave a greater number of major peaks than sample SOA76-173, indicating a compositional difference between these two fractions. Similar comparisons were made with the other shale oil samples investigated. The relative fluorescence intensities for the major fluorescence peaks in the 5-ring PAH fractions for four shale oil samples are compared in Figure 7; also shown are R_f ranges for fluorescence peaks and possible compound types and their R_f values. Sample 18 (SOA79-1) shows the highest concentration of 5-ring PAH.

Table 5 lists the R_f values for the PAH standards obtained with the 30%-acetylated cellulose TLC system employed. For the 6-, 5-, and 4-ring

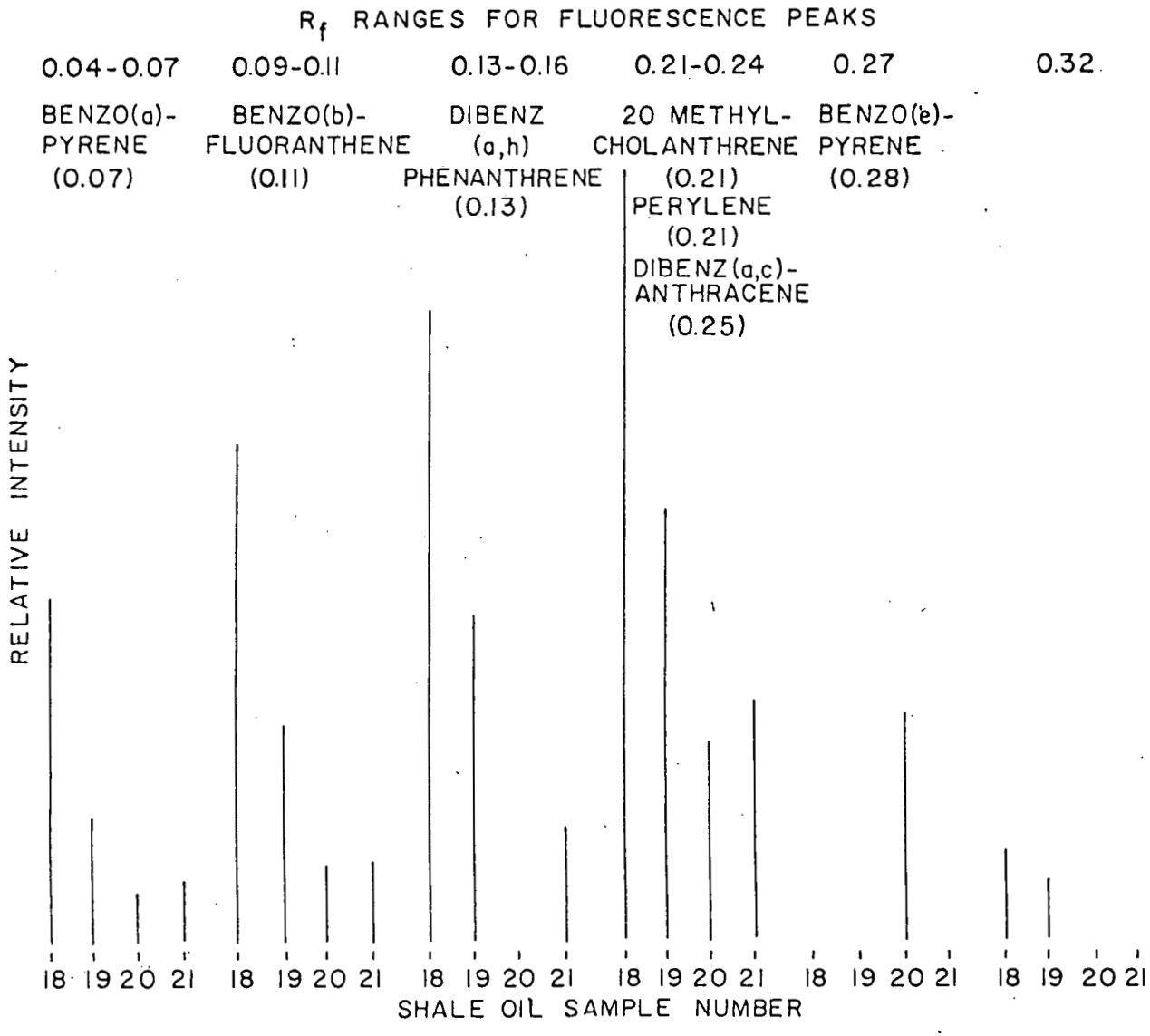


Figure 7. Comparison of 5-ring PAH in four shale oil samples, with R_f ranges for fluorescence peaks, possible compounds and their R_f values. (See Table 3 for sample number information.)

PAH, no R_f values exceed 0.33; for the 3-ring PAH, no R_f values exceed 0.43. All the fluorescence profiles obtained for the different ring fractions indicated fluorescence components beyond these R_f limits (Table 4). This suggested that the fluorescence beyond the R_f limits was not due to PAH but mainly to nitrogen heterocycles.

TABLE 5

R_f values for polycyclic aromatic hydrocarbons

Compounds	R_f	Compounds	R_f
6-Ring Systems		4-Ring Systems	
3,4,8,9-Dibenzpyrene	0.02	Chrysene	0.11
3,3,9,10-Dibenzpyrene	0.10	9,10-Dimethyl-1,2-benzanthracene	0.21
2,3-o-Phenylenepyrene	0.10	1,2-Benzofluorene	0.25
1,2,4,5-Dibenzyprene	0.19	Pyrene	0.24
1,2,7,8-Dibenzchrysene	0.22	Triphenylene	0.29
Coronene	0.22	2,3-Benzofluorene	0.31
1,2,3,4-Dibenzpyrene	0.31	Fluoranthene	0.33
5-Ring Systems		3-Ring Systems	
Benzo[a]pyrene	0.07	Phenanthrene	0.34
1,2,6,7-Dibenzphenanthrene	0.13	9,10-Diphenylanthracene	0.34
3,4-Benzofluoranthene	0.11	1-Methylphenanthrene	0.40
20-Methylcholanthrene	0.21	2-Methylphenanthrene	0.40
Perylene	0.21	2-Methylanthracene	0.36
1,2,3,4-Dibenzanthracene	0.25	9-Methylanthracene	0.33
Benzo[e]pyrene	0.28	9,10-Dimethylanthracene	0.36
		Anthracene	0.38
		Fluorene	0.43
		3,6-Dimethylphenanthrene	0.43

Nitrogen Heterocycles

Aluminum oxide dry-column experiments with carbazole-type and pyridine-type standards and n-hexane-ether (19:1) as the mobile phase showed that some of these compounds migrated on the column. Carbazole and 1,2-benzocarbazole did not migrate, but 9-methylcarbazole (distance ≈ 8.5 cm), 9-ethylcarbazole (≈9.2 cm), and 9-n-butylcarbazole (≈10.8 cm) did migrate. Benzo[c]cinnoline, 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline and 1,10-phenanthroline monohydrate did not migrate. 2,2′-Biquinoline (≈1 cm), acridine (≈1 cm), phenazine (≈0.7 cm), 5,6-benzoquinoline (≈0.5 cm), phenanthridine (≈0.5 cm) migrated slightly. Bender and Elbert [11] have indicated the R_f values of several pyridines in an aluminum oxide-pentane-

ether (19:1) system; they commented that compounds with a non-sterically-hindered aza nitrogen are attracted more strongly to aluminum oxide than compounds with a sterically-hindered aza nitrogen and thus have low R_f values. Sterically-hindered pyridines can migrate at rates similar to PAH. From some experiments done here with Al_2O_3-n-pentane-ether (19:1) and the results given by Bender and Elbert, it can be concluded that carbazoles substituted in the 9-position, and sterically-hindered pyridines, can migrate with PAH. It appears that the fluorescence beyond the R_f limits for PAH discussed above could be due to such compounds. The migration distances of compounds such as 2,2´-biquinoline and acridine on an alumina column indicated that these compounds could appear in the 6-ring PAH fraction. The migration distances on alumina columns for the 9-alkyl-substituted carbazoles indicated that they could appear in the 3-,4-, or 5-ring PAH fractions. Table 6 gives the R_f values for several carbazole and pyridine types on the acetylated-cellulose TLC system employed. The R_f values show that compounds of these types can migrate readily. All the compounds in Table 6 except 2,2´-biquinoline and 9-methylcarbazole have R_f values greater than the R_f limit for 6-, 5-, and 4-ring PAH, namely, 0.33. Also, several of the compounds in Table 6 have R_f values greater than 0.43, the R_f limit of 3-ring PAH. No spectral work was done to identify the components beyond the R_f limits mentioned above.

TABLE 6

R_f values for nitrogen heterocycles

Compound	R_f	Compound	R_f
3-Methylcarbazole	0.42	1,10-Phenanthroline Monohydrate	0.77
9-Methylcarbazole	0.33	Phenazine	0.69
9-Ethylcarbazole	0.41	2,9-Dimethyl-4,7-diphenyl-1,-10-phenanthroline	0.55
9-n-Butylcarbazole	0.38		
1,2,3,4-Tetrahydrocarbazole	0.76	4-Azafluorene	0.62
1,2,7,8,-Dibenzocarbazole	0.36	5,6-Benzoquinoline	0.61
7H-Dibenzo(c,g)carbazole	0.23	Acridine	0.65
Carbazole	0.39	Dibenz(a,j)acridine	0.39
2,2´-Biquinoline	0.24		

Removal of Pyridine Types

Results obtained in this work and previous work showed that many pyridine compounds did not migrate with a chloroform mobile phase on a silica gel column that had concentrated hydrochloric acid absorbed at the top of the column [12]. The exact mechanism for the inhibition of migration of the pyridine compounds is not known; presumably the compounds are protonated at the ring nitrogen and are thus strongly adsorbed onto silica gel. In this work it was shown that acridine, 4-azafluorene, benz(c)acridine, 2,2´-biquinoline and 2,9-dimethyl-4, 7-diphenyl-1,10-phenanthroline did not migrate or migrated only slightly on the acidified silica gel column with chloroform as a mobile phase. Carbazole compounds migrated readily on the acid silica gel column. Another separation step was added to remove pyridine compounds from the shale oil samples. With this additional separation step, the overall separation scheme involved an acid silica gel step, a dry-column aluminum oxide step, and finally an acetylated-cellulose TLC step.

Shale oil samples SOA76-173 and SOA77-205 were investigated further with the additional separation step. This was done by carrying out the separation procedure twice, once with the acid silica gel step and the other without this step. In this way, two fluorescent profiles were obtained. One profile represented PAH and some nitrogen heterocycles, and the other PAH with most pyridine compounds removed. Additional work is needed to define other classes of compounds in the fractions, but the results reported here indicate that mainly nitrogen heterocycles and PAH are found in the fractions without the acid silica gel step included.

The fluorescence profiles obtained for the 4- and 3-ring PAH fractions from sample SOA77-205 with the acid silica gel step included are compared in Figures 8 and 9. The fluorescence beyond the R_f limits discussed earlier is absent. Without the acid silica gel step, the fluorescence remains beyond the R_f limits, as shown in Figures 4 and 6. These results and the results in Tables 5 and 6 indicate that some nitrogen heterocycles are responsible for the fluorescence beyond the R_f limits for PAH. The fluorescence beyond these limits is probably due to carbazole types and sterically-hindered pyridine compounds. More work is needed to characterize and identify these nitrogen heterocycles.

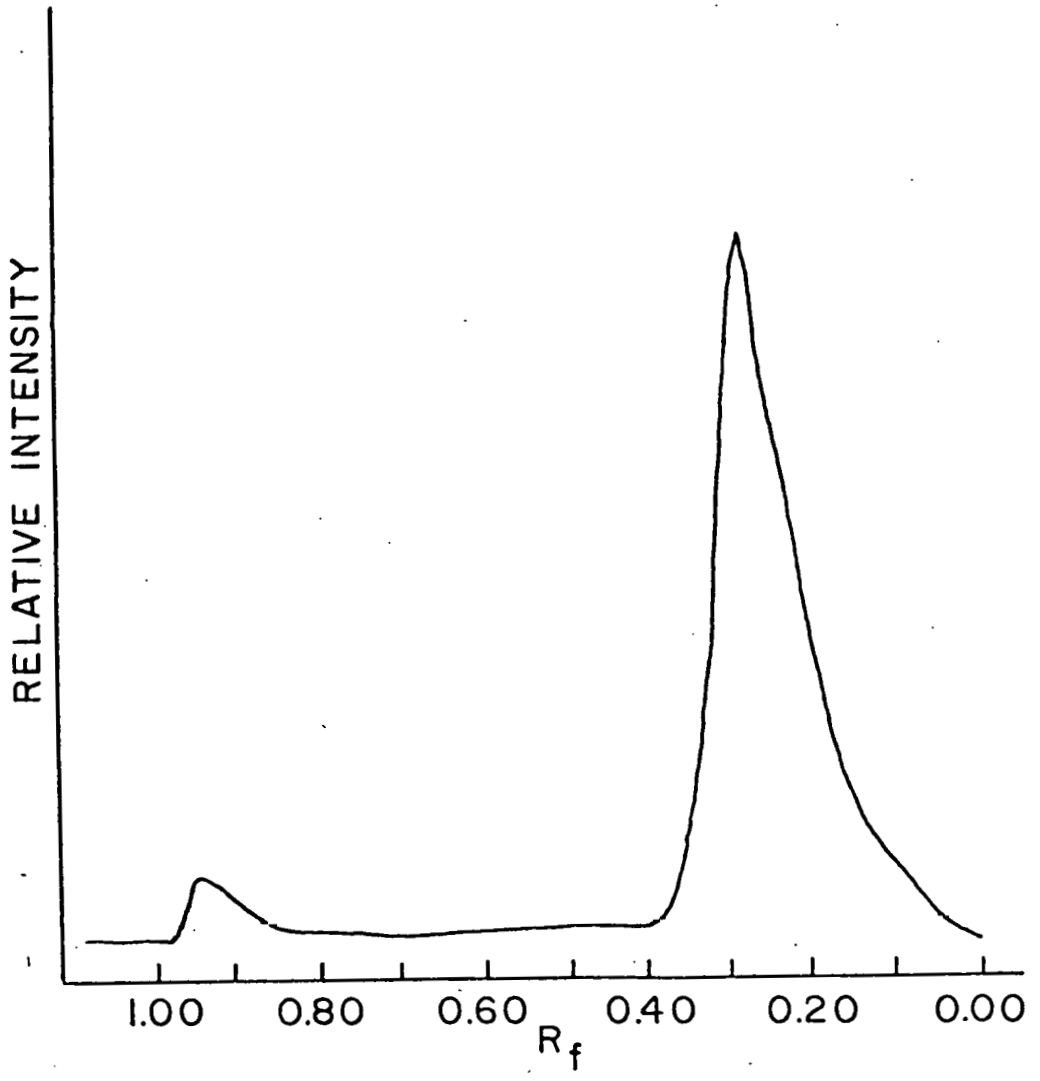


Figure 8. Fluorescence profile of 4-ring fraction from sample SOA77-205 with acid treatment.

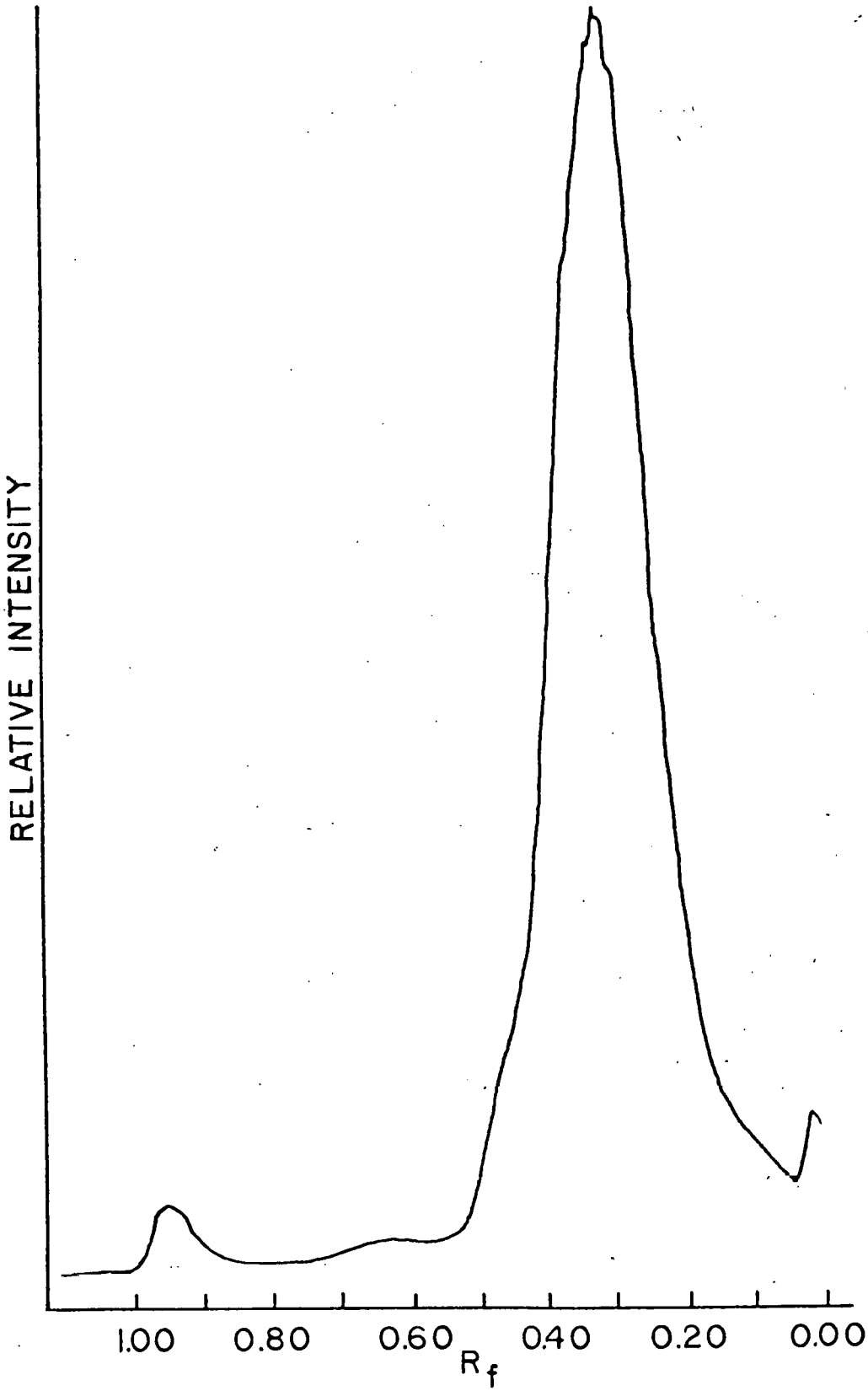


Figure 9. Fluroescence profile of 3-ring fraction from sample SOA77-205 with acid treatment.

Percentage Recovery of Benzo[a]pyrene

As a general check on the percentage recovery of PAH for the characterization procedures with and without the acid silica gel step, the percentage recovery benzo[a]pyrene (BaP) was determined by a method developed previously [4]. Samples (4 μg) of BaP were added separately to an acidified silica gel column, an alumina column and a shale oil sample. The acidified silica gel column was eluted with chloroform, the alumina column was eluted with n-hexane-ether (19:1), and the spiked shale oil sample was run through both an acidified silica gel-chloroform system and alumina-n-hexane-ether (19:1) system. Finally, the BaP was determined by measuring the fluorescence reflected from the acetylated cellulose as described earlier [4]. For the acidified silica gel column, the recovery was 102%, for the alumina column the recovery was 98%, and for the shale oil sample the recovery was 103%. BaP was also determined in several shale oil samples with and without the acid silica gel step; the results are listed in Table 7. All the results indicated that BaP can be determined acurrately with the acid silica gel step included.

TABLE 7

Determination of benzo[a]pyrene

Sample	Concentration of benzo[a]pyrene (ppm)[a]	
	Without acid silica gel step	With acid silica gel step
SOA76-173	28	31
SOA77-205	64	66
SOA76-135	7.2	8.9
SOA77-231	11	9.2
SOA77-233	14	15

[a]Duplicate determinations.

EXPERIMENTAL

Apparatus, reagents and materials

All fluorimetric measurements were made with a Schoeffel SD3000 spectrodensitometer in the reflection mode. Only an ultraviolet cutoff filter was employed in the reflection mode so that fluorescence emitted throughout the visible region was detected.

Individual PAH and other samples were obtained from commercial sources and were recrystallized when needed. The shale-oil samples were obtained from the Laramie Energy Technology Center, Laramie, Wyoming.

The 30% acetylated cellulose thin-layer chromatoplates were Brinkmann precoated Cel 300 AC/30-20 plates. The aluminum oxide (activity II-III) for dry-column chromatography was obtained from ICN Life Science Group, Cleveland, Ohio. The silica gel 60 (MN-Kieselgel 60 0.1-0.2 mm/70-140 mesh ASTM) was obtained from Brinkmann. Nylon lay-flat tubing was obtained from Hall Manufacturing Corp., Mahwah, New Jersey. A 10-µl Hamilton syringe was used in spotting the chromatoplates. A Buchi Rotavapor-M was used for evaporation of solvents.

Procedures

Characterization without acid treatment. A 28-cm section of nylon lay-flat tubing (i.d., 6.62 mm) plugged with glass wool at one end was packed with 8.50 g of aluminum oxide. The height of the aluminum oxide was ca. 17.5 cm. Aluminum oxide (0.5g) was added to a 30-ml evaporating flask. Then a 0.0500 g sample of shale oil was weighed accurately in the flask and 1 ml of n-hexane added. The mixture was heated to 30°C in the flask while stirring under vacuum with a rotary evaporator. This step assured that the

shale oil sample was distributed evenly on the aluminum oxide. The dried aluminum oxide with shale oil adsorbed on it was transferred quantitatively to the top of the aluminium oxide already in the column. The mobile phase (1 ml), n-hexane-ether (19:1) was used to rinse the evaporating flask and the rinsings were quantitatively transferred to the column. After this volume had absorbed into the aluminum oxide, 6 ml more of n-hexane-ether (19:1) was added to the column. After development of the column, it was cut into four sections. The lengths of each successive section starting from the interface of the aluminum oxide that had shale oil initially adsorbed and fresh aluminum oxide were 0.0-6.5 cm, 6.5-11.5 cm, 11.5-15.0 cm, and 15.0-20.0 cm. Later work with nylon tubing of 7.18 mm i.d. gave section lengths of 0-4 cm, 4-8 cm, 8-12.5 cm, and 12.5-17 cm. The sections contained maximum concentrations of 6-, 5-, 4-and 3-ring PAH, respectively, though fluoranthene overlapped into the 3-ring fraction. With a given diameter of tubing and alumina activity, migration rates were very reproducible. When these factors changed, a standard column was run to establish new migration distances. An identical standard aluminum oxide column was prepared that had coronene, benzo[a]pyrene, triphenylene, fluoranthene, phenanthrene, and fluorene absorbed on the top of the column. This column was developed in an identical manner as the sample column and then the sample column was cut into sections based on the migration distances of the standards. The aluminum oxide sections were stirred separately in beakers for 20 min with 10 ml of 1,2-dichloroethane and then the 1,2-dichloroethane was decanted. The aluminum oxide sections were washed with 5 ml of 1,2-dichloroethane, and the combined extracts from each section were evaporated individually to dryness by directing air into beakers heated at 30°C. A small amount of n-hexane

was added to each beaker and then the n-hexane aliquots were transferred quantitatively to a 1-ml volumetric flask. The beakers were rinsed with additional aliquots of n-hexane, the rinsings were added to the appropriate volumetric flask, and the final volume was adjusted to 1 ml.

Each solution was spotted (5 µl) 2.0 cm from the bottom edge of a 30% acetylated cellulose chromatoplate that had been cleaned previously by development with methanol-n-hexane-acetone (20:5:3:3) which had been allowed to equilibrate for 1 h before development of the chromatoplate. After development, the chromatoplate was dried in air and then positioned on the stage of the spectrodensitometer. The excitation monochromator was set at 310 nm. The stage of the spectrodensitometer was started and the visible fluorescence of the components distributed on the chromatoplate recorded. Scans were made from 1 cm above the solvent front to the spot origin.

Characterterization with acid treatment. A 0.25-g quantity of silica gel was added to a 30-ml evaporating flask. Then an accurately weighed 0.0500-g sample of shale oil was weighed in the flask and 1 ml of diethyl ether added. The mixture was heated to 30°C in the flask while stirring under vacuum with a rotary evaporator. After evaporation of the diethyl ether, the silica gel with shale oil absorbed on it was transferred quantitatively to a previously prepared acid-treated silica gel column. Chloroform (1 ml) was used to rinse the evaporating flask and the rinsings were added to the column. The acid-treated silica gel column was prepared by slurry-packing 3.30 g of silica gel in distilled chloroform into a 15 cm x 1.05 cm i.d. glass column. Then 0.2 ml of concentrated hydrochloric acid was allowed to absorb into the silica gel at the top of the column [12]. The acid-treated silica gel with shale oil was eluted with 9 ml of chloroform,

and the chloroform was collected in a 30 ml evaporating flask that contained 0.5 g of aluminum oxide. This sample was then heated to 30°C in a rotary evaporator and transferred to the aluminum oxide column as described above.

D. ROOM TEMPERATURE PHOSPHORESCENCE (RTP) OF NITROGEN HETEROCYCLES

The room temperature phosphorescence (RTP) work was partially supported under this contract. Eight nitrogen heterocycles were studies in detail for their potential to yield strong RTP. The research involved determining the best experimental conditions for RTP, designing and constructing a phosphoroscope, discovering and explaining the interactions needed to induce RTP, and applying RTP for the identification of nitrogen heterocycles in shale oil.

Analytical Conditions and Data

To induce RTP from nitrogen heterocycles such as benzo[f]quinoline (B[F]Q) and other compounds, it was necessary to adsorb the compounds on solid surfaces such as filter paper or silica gel. (C.D. Ford and R.J. Hurtubise, Anal. Chem. 51, 659 (1979.) RTP and fluorescence analysis of compounds adsorbed on solid surfaces such as silica gel and filter paper are powerful qualitative and quantitative analytical tools because of their sensitivity and selectivity. The phosphoroscope discussed in "Experimental" at the end of this section, and designed for use with a Schoeffel SD3000 spectrodensitometer, works well for obtaining RTP from compounds adsorbed on silica gel chromatoplates or filter paper. Furthermore, silica gel was stressed over filter paper in the RTP work because RTP can be combined with the speed and versatility of TLC on silica gel to yield a very useful analytical approach for determining nitrogen heterocycles. Certainly, silica gel or filter paper can be used in conjunction with high-performance liquid chromatography (HPLC) by collecting a fraction from a HPLC column, adsorbing the components on the solid surface, and then measuring either fluorescence or RTP.

Silica Gel

RTP was observed from compounds adsorbed on aluminum backed and glass backed, conventional TLC and HPTLC silica gel plates (EM Laboratories). No RTP was observed from the compounds adsorbed on Brinkmann (MN) plastic backed silica gel TLC plates. RTP was also observed from compounds adsorbed on dried MN-Kieselgel 60 (Düren, Germany) for dry column chromatography; however, no RTP was observed for compounds adsorbed on dried Mallinckrodt SilicAR TLC-7G for TLC.

Excitation and Emission Spectra

The phosphorescence excitation and emission spectra of each compound adsorbed on dried silica gel were obtained using the phosphoroscope accessory of the MPF-2A fluorescence spectrophotometer. The uncorrected phosphorescence excitation and emission spectra of B[f]Q were obtained at liquid nitrogen temperature (LTP) with the protonated compound in ethanol and at room temperature with the protonated compound adsorbed on a dried aluminum backed silica gel chromatoplate (Figure 10). The LTP excitation spectrum of B[f]Q gave peaks at 287 and 367 nm, and the RTP excitation spectrum gave peaks at 291 and 367 nm. The LTP emission spectrum of the compound in ethanol showed peaks at 437, 475, and 509 nm, whereas, the RTP emission peaks were at 434, 488, and 516 nm.

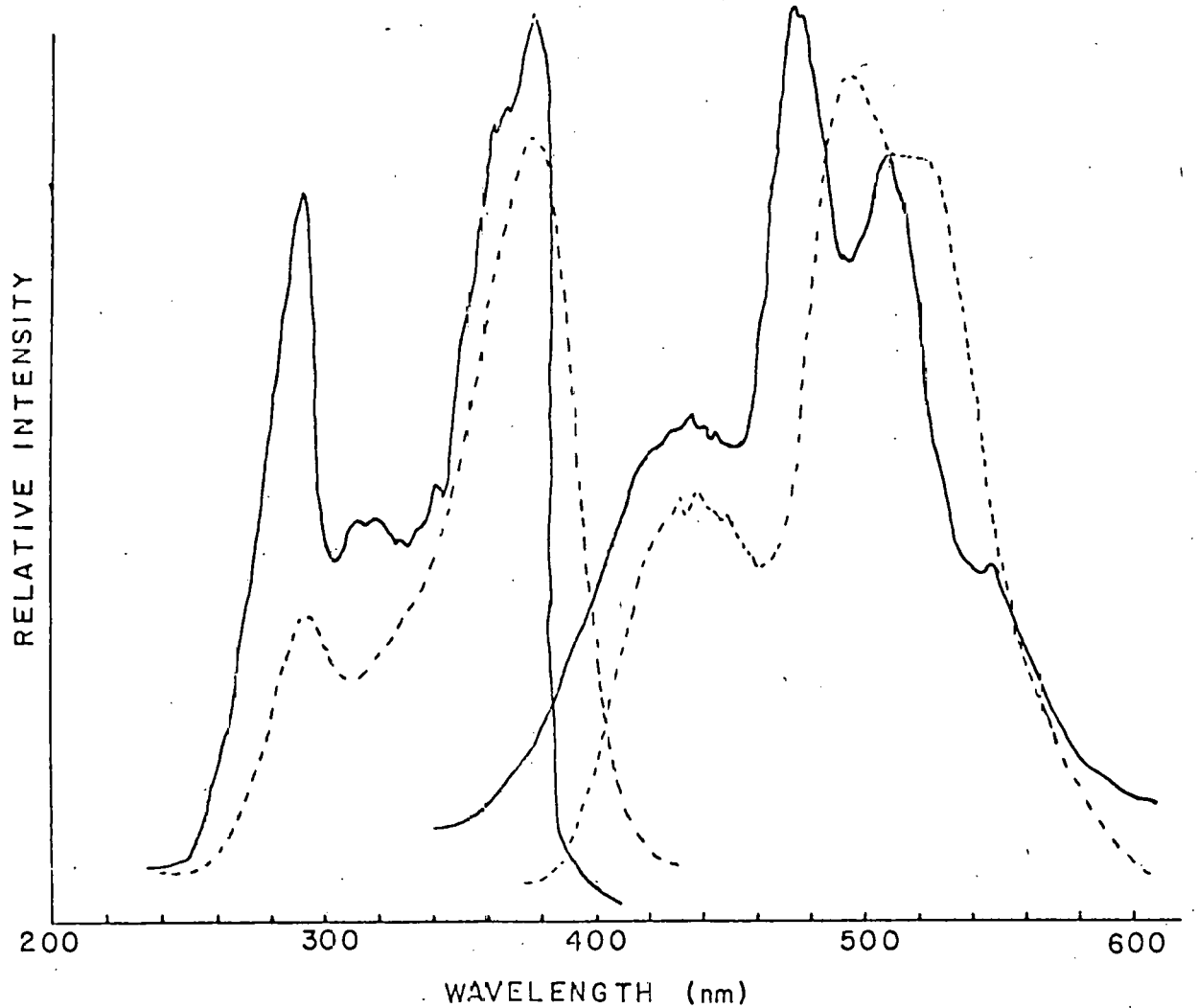


Figure 10. The LTP (——) and RTP (---) excitation and emission spectra of B[f]Q in ethanol (LTP) and on dried silica gel (RTP). Phosphorescence intensities were adjusted arbitrarily.

Acid Effect on the RTP of Nitrogen Heterocycles.

The LTP excitation and emission spectra of phenanthridine were obtained both in ethanol and in acidified ethanol (0.1 M HCl) with the fluorescence spectrophotometer. A comparison of the spectral data showed a red shift in the emission spectra in going from ethanol solution to 0.1 M HCl ethanol solution of the compound. The LTP intensity was approximately doubled in going from an ethanol solution to an acidified ethanol solution of phenanthridine at a concentration of 1.0 μg/mL. These data suggest that protonation of the nitrogen heterocyclic compound serves to enhance the LTP.

Ethanol solutions with a fixed amount of B[f]Q containing from 10^{-6} to 1.0 M HCl were spotted in duplicate on a silica gel chromatoplate and the plate was dried. The RTP of B[f]Q vs. the concentration of HCl plot appeared as an S-shaped curve. Graphs of the data obtained for phenanthridine and 1,10 phenanthroline were similar to that for B[f]Q with the increase in RTP occurring between 10^{-3} and 10^{-1}M HCl. Therefore 0.1 M HCl ethanolic solutions of the nitrogen heterocycles were employed for the quantitative RTP data of these compounds adsorbed on solid surfaces.

Table 8 gives a comparison of the relative RTP intensities of the nitrogen heterocycles. The compounds were spotted from ethanol solutions and ethanol solutions which were 0.1 M in HCl onto silica gel and filter paper and measured in air. The data in Table 8 show that in each case protonation of the nitrogen heterocycle enhances the RTP of the compounds adsorbed on dried silica gel and filter paper. Just as other workers have established that anion and cations of certain organic compounds exhibited more intense RTP on solid surfaces than the neutral compound, it seemed

reasonable that the cations of the nitrogen heterocycles should exhibit more intense RTP when adsorbed on solid surfaces than their neutral counterparts (13-15).

TABLE 8

Comparison of RTP Data of N-Heterocycles Spotted on Silica Gel and Filter Paper

Compound[a]	λex, nm	λem, nm[b]	silica		filter paper	
			neutral RI[c]	acid RI[d]	neutral RI[c]	acid RI[d]
benzo[f]quinoline	370	510	5.1	55.2	20.5	100
phenanthridine	360	510	3.6	42.4	8.5	46.0
4-azafluorene	330	465	6.4	22.8	17.5	42.0
1,10-phenanthroline-H_2O	325	520	1.8	7.5	7.5	17.5
4,7-diphenyl-1,10-phenanthroline	310	518	N.D.[e]	11.3	4.5	23.5
2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline	330	518	N.D.	12.0	13.5	17.5
quinoline	320	510	N.D.	7.0	7.5	20.0
isoquinoline	330	520	2.0	8.0	3.7	25.0

[a]Each compound at 100 ng/spot. [b]λ_{ex} and λ_{em} maximized for Schoeffel unit. [c]Compounds spotted from ethanol solutions. [d]Compounds spotted from ethanol solutions which were 0.1 M HCl. [e]N.D. = non-detected.

Analytical Data

Table 9 gives the linear range of the RTP calibration curves obtained from the compounds adsorbed on dried silica gel and the RTP limits of detection (LOD = ng/spot which gives 2X the background signal). The data are given for the RTP of the protonated compounds measured in air. In no case did measuring the RTP of the compounds spotted from acid solutions on dried silica gel under N_2 or He flow alter the linear ranges, only the LODs changed slightly. However, the data scatter of some of the weaker phosphors with N_2 and He was less than for air.

TABLE 9

RTP Analytical Data from Compounds Adsorbed on Dried Silica Gel[a]

Compound	λ_{ex}, nm	λ_{em}, nm[b]	linear range, ng	LOD, ng/spot
benzo[f]quinoline	370	510	0-125	3
phenanthridine	360	510	0-160	6
4-azafluorene	330	465	0-100	10
1,10-phenanthroline-H_2O	325	520	0-140	25
4,7-diphenyl-1,10-phenanthroline	310	518	0-175	12
2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline	330	518	0-150	20
quinoline	320	510	0-100	25
isoquinoline	330	520	0-250	22
p-aminobenzoic acid	287	432	0-150	10
2,5-pyridine-dicarboxylic acid	289	435	0-120	15

[a]All compounds spotted from ethanol solutions which were 0.1 M HCl except p-aminobenzoic acid and 2,5-pyridine dicarboxylic acis which were spotted from ethanol solutions. [b]λ_{ex} and λ_{em} were maximized for the Schoeffel unit.

Reproducibility of RTP

Sixteen spots of B[f]Q from 0.1 M HCl ethanol solution at 100 ng each were placed on a silica gel chromatoplate, and the RTP from each spot was recorded in air as described in Experimental. The standard deviation was determined to be 1.50 relative intensity (RI) units. The 95% confidence limits were calculated to be 46.5 ± 0.8 RI units. A similar experiment with 4-azafluorene resulted in a standard deviation of 0.93 RI units and 95% confidence limits of 16.7 ± 0.5 RI units for sixteen 100-ng spots.

Plate-to-Plate Variation of RTP

Three aluminum backed silica gel chromatoplates were each spotted with five 100-ng spots of B[f]Q. After drying, the RTP signals were recorded for each with the modified spectrodensitometer. The average RTP

intensities obtained for the three plates were 62.0 (plate 1), 54.8 (plate 2), and 57.6 (plate 3). Because of the plate-to-plate variation in RTP of compounds adsorbed on silica gel, any quantitative method which employs RTP from compounds adsorbed on commercial chromatoplates should include RTP measurement of the proper standards along with the unknowns on the same chromatoplate.

Total Luminescence Approach

Several of the compounds in Table 9 also exhibited visible fluorescence when adsorbed on dried silica gel. For example, B[f]Q, phenanthridine, and quinoline exhibit visible fluorescence in addition to RTP when adsorbed on dried silica gel. The fluorescence emission maxima for the three compounds were 428, 418, and 400 nm, respectively. There are certain analytical advantages to employing both fluorescence and RTP. For example, the identification of unknowns is aided by fluorescence excitation and emission spectra, RTP emission spectra, and the RTP lifetime of compounds that can be measured easily. Also, one is not restricted to a single luminescence process. If, for example, B[f]Q were being determined and the spot containing B[f]Q was found to contain a fluorescent impurity but no phosphorescent impurity, RTP could be employed to quantitate the B[f]Q in the sample without interference from the fluorescent impurity.

A fluorescence calibration curve was obtained for B[f]Q adsorbed on dried silica gel in air. The linear range was found to be 0-100 ng and the limit of detection was 1 ng. When dry He was blown over the spot, the fluorescence signal was enhanced by 30%, indicating some O_2 quenching with air.

EXPERIMENTAL

Apparatus.

Phosphorescence excitation and emission spectra were obtained with a Perkin-Elmer MPF-2A fluorescence spectrophotometer equipped with a phosphoroscope accessory. A 150-W Xe arc lamp and a 1P28 photomultiplier tube were employed. RTP spectra were recorded with the excitation and emission slits of the fluorescence spectrophotometer set at 12 and 14 nm, respectively, directly from 100 ng of the compound adsorbed on a 4 cm by 0.8 cm section of a dried aluminum backed silica gel chromatoplate. The chromatoplate section was placed at approximately a 45° angle with respect to the source and the emission slit. The mounting device which held the chromatoplate was constructed of two 4 cm x 0.5 cm x 0.5 cm aluminum bars which were drilled and tapped at opposite ends so the chromatoplate section could be mounted between the metal bars. With the chromatoplate section thus fixed, the device was mounted on the support frame of the rotating can phosphoroscope and the RTP signal from the compound adsorbed on the silica gel plate was maximized.

Calibration curves and relative RTP signals were measured with a Schoeffel SD3000 spectrodensitometer with the inlet and exit slits at 2 and 3 mm, respectively. A 150-W Xe lamp and R-777 photomultiplier tube were employed.

Because some of the compounds studied exhibited fluorescence which interfered with RTP measurements, it was necessary to modify the spectrodensitometer and to design and construct a reflection mode assembly and a phosphoroscope which would be compatible with the Schoeffel spectrodensitometer. The assembly allowed the distance from the source exit

to the photomultiplier tube to be varied by means of an adjustable slide. Also, the angle of the photomultiplier tube housing could be adjusted to maximize the reflected RTP striking the lens of the photodetector system. The above modifications were necessary to accommodate a rotating disc phosphoroscope.

The phosphoroscope assembly consisted of a variable speed dc motor (0-12V) and a rotating disc phosphoroscope. The motor was powered by a Railine model 370N transformer. The assembly was designed for use with the modified reflection mode assembly of the spectrodensitometer. The phosphoroscope was constructed of thin sheet aluminum and was painted flat black to minimize scattered radiation. The dc motor was mounted in an aluminum frame which allowed the phosphoroscope to be raised or lowered via an adjustable slide. Also, the angle of the photomultiplier tube housing could be adjusted to maximize the reflected RTP striking the lens of the photodectector system. The above modifications were necessary to accommodate a rotating disc phosphoroscope.

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inert gases over the adsorbed compounds. The copper tubing (5 mm o.d.) was 24.5 cm in length and plugged at one end. About 1 cm from the plugged end; there was a small slit out of which the inert gases flowed onto the adsorbed compounds. The copper tubing was mounted in a lead block for stability. The gases (N_2 and He) were passed through a drying tube (a plastic tube containing $CaSO_4$) before being forced out the tube and over the adsorbed compounds during the measurement step.

Reagents

Ethanol was purified by distillation. Benzo[f]quinoline (B[f]Q) and phenanthridine were recrystallized from ethanol and distilled water. Quinoline was distilled at reduced pressure with a 61-cm bubble plate fractionation column and collected at 54°C. Both 2,5 pyridine dicarboxylic acid and p-aminobenzoic acid were recrystallized from ethanol, and 4-azafluorene was recrystallized from hexane, ethanol, and distilled water. Each compound was checked for luminescent impurities by TLC and, if no impurities were observed, the compounds were used without further purification. All other compounds were reagent grade and used as received. The silica gel chromatoplates were aluminum backed (EM Laboratories, Elmsford, N.Y.) and were developed in distilled ethanol prior to RTP experiments to concentrate any luminescent impurities at one end of the plate. The filter paper employed was Whatman No. 1 and was used as received.

Procedure

All compounds were dissolved initially in distilled ethanol prior to spotting on a silica gel chromatoplate or filter paper. For calibration

curves, each compound except p-aminobenzoic acid and 2,5 pyridine dicarboxylic acid was spotted from ethanol solutions which were 0.1 M HCl. Each solution containing a nitrogen heterocycle was acidified by mixing 40 µL of concentrated HCl with 5 mL (final volume) of the ethanol solution to be spotted. The acid was added by means of a Quickpipette (Helena Labs, Beaumont, Tex.)

Careful attention was given to the spotting technique employed for RTP work. Prior to spotting, the plates or filter papers were marked with a pencil so straight dashed lines appeared in the same plane across the solid surface. The Hamilton syringe containing the adsorbate solution was positioned carefully between dashes so the center of each spot lay in line with the markings. Best reproducibility was realized when spot size was maintained as constant as possible. Of the 3 µL spotted, one third of the solution was initially placed on the plate or filter paper. Care was taken not to increase the size of this initial spot while spotting the remaining two thirds of the sample solution. With some practice, this technique gave good reproducibility.

After spotting the silica gel chromatoplate in duplicate with 3 µL of each fresly prepared standard solution with a 5-µL Hamilton syringe, the aluminum backed plate was allowed to dry in the atmosphere for 5 min and was then placed in an oven at 105-110°C to dry for an additional 30 min. The plate was removed from the oven and allowed to cool to room temperature. Then the plate was positioned carefully on the stage of the densitometer. The RTP signal was maximized for the most concentrated spot, and the signals for all the spots were measured by starting the moving stage of the densitometer. It was not necessary to maximize the

signal for each spot becasue of the careful spotting technique employed.

When filter paper was employed as the adsorbent, 3 μL of the solution containing the nitrogen heterocycle was placed on the paper in duplicate, again using a 5-μL Hamilton syringe. The filter paper was allowed to dry in the atmosphere for 5 min before being placed in an oven to dry for 10 min at 105-110°C. If the paper was placed in the oven immediately after spotting, the spots of the compound which were acidified appeared blackened after drying. After drying, the paper was placed on the stage of the modified spectrodensitometer and the RTP signals were recorded as described above for silica gel plates. No special drying procedure was performed during the measurement step for the data in Table 8 and 9.

Interactions responsible for RTP

The room temperature phosphorescence (RTP) of B[f]Q adsorbed on several silica gel samples was investigated by luminescence, reflectance, and infrared spectroscopy to obtain a better understanding of the analytical conditions needed for strong RTP. The results showed that silica gel chromatoplates containing a polymeric binder with carboxyl groups were the best samples for inducing strong RTP from B[f]Q. The polymer itself was essential for inducing strong RTP. It was postulated that B[f]Q was adsorbed flatly on the surface with the carboxyl groups anchoring via hydrogen bonding with π electrons. These same interactions should be operative for other nitrogen heterocycles. Full details of this study will be published in Analytical Chemistry in the early part of 1980. Only some of the results will be given in this report.

Table 10 lists those brands of silica gel which were tested as surfaces for B[f]Q and indicates the relative magnitude of the RTP signal observed for B[f]Q.

TABLE 10

The Silica Gel Brands Tested as RTP Supports for B[f]Q

Brand	Description	RTP (Neutral)[b]	RTP (Acid)[c]
EM[a]	Al backed TLC Chromatoplate	moderate	strong
EM	Glass backed TLC Chromatoplate	moderate	strong
EM	Plastic backed TLC Chromatoplate	moderate	strong
EM	Glass backed (HPTLC) Chromatoplate	moderate	strong
Brinkmann	Plastic backed N-HR (TLC) Chromatoplate	moderate	strong
Brinkmann	Plastic backed Sil-G (TLC) Chromatoplate	none	none
S & S	Glass backed (TLC) Chromatoplate	none	moderate
Applied Science Labs	Glass backed Permakotes I (TLC) Chromatoplate	none	weak
EM	Silica Gel 40, Column Chromatography	none	none
EM	Silica Gel 60, Column Chromatography	none	none
EM	Silica Gel 100, Column Chromatography	none	none
MN[d]	Silica Gel 60, Column Chromatography	none	weak
MN	Kieselgel 60, for TLC Chromatography	none	weak
Mallinckrodt	SilicAR TLC-7G Chromatography	none	none

[a]All work done with aluminum backed EM Chromatoplate unless stated otherwise in the text. [b]B[f]Q spotted from ethanol. [c]B[f]Q spotted from 0.1 M HCl ethanol. [d]Macherey, Nagel & Co.

There was a 2.4-fold increase in the RTP of B[f]Q spotted from HBr ethanol solution compared to B[f]Q spotted from HCl-ethanol solution. The results suggest, in addition to the acid effect, some of the RTP enhancement can be attributed to the heavy atom, Br^-. The RTP lifetime of the HBr spot of B[f]Q was noticeably shorter than the HCl spot of B[f]Q on an EM silica gel chromatoplate. Also, the fluoresecence of the compound from HBr solution on the chromatoplate was less intense than the compound spotted from HCl solution. The use of HBr for the enhancement of the RTP of nitrogen heterocycles should be further studies and developed to its fullest analytical potential.

Infrared data and other information indicated that the polymeric binder used in EM chromatoplates was the sodium salt of polyacrylic acid. Experiments were performed in which polyacrylic acid was mixed with MN silica gel 60 for column chromatography to give mixtures containing varying percentages of polyacrylic acid. B[f]Q exhibited a weak RTP when adsorbed on MN silica gel (Table 10). These mixtures were placed in depressions in a blackened brass plate (15). On each of these silica gel samples, 600 ng of B[f]Q were spotted from 0.1 M HCl ethanol solution with a 5 µl Hamilton syringe. After drying, the RTP relative intensity of B[f]Q on each sample was obtained. The data is given in Table 11. From the data in Table 11, it can be seen the RTP signals increased almost linearly to 10% polyacrylic acid and decreased above 20% polyacrylic acid. The data in Table 11 also indicated 100% polyacrylic acid is not a good surface for inducing RTP. The above seems to indicate that a certain percentage of polyacrylic acid is important in achieving the strong adsorbate-solid surface interaction which induces enhanced RTP.

To determine if polyacrylic acid could be mixed with other inorganic solids and remain a suitable RTP surface, mixtures of polyacrylic acid and crushed NaCl were prepared. NaCl was chosen because no RTP was observed from B[f]Q adsorbed on NaCl. The RTP intensities were measured for 600 ng of B[f]Q spotted from 0.1 M HCl ethanol solution. The RTP data paralleled that of the polyacrylic acid - MN silica gel mixtures, in that the phosphorescence increased to 20% polyacrylic acid and decreased above 20% polyacrylic acid. However, the intensity of the RTP of B[f]Q adsorbed on polyacrylic acid - NaCl mixtures was about twice that of the compound on polymer-MN silica gel mixtures when compared at 5% polyacrylic acid (Table 11).

TABLE 11

The RTP Relative Intensities of 600 ng of B[f]Q Spotted on Samples Containing Varying Percentages of Polyacrylic Acid in MN Silica Gel and NaCl

%Polyacrylic Acid	Relative Intensity With MN Silica Gel[a]	Relative Intensity with NaCl[a]
0	1.0	0.0
5	21	42
10	43	56
20	49	67
30	31	48
100	2.3	2.3

[a]Average from duplicate spots

The detailed study indicated that hydrogen bonding was very important for inducing RTP. In addition, polyacrylic acid binder was essential for inducing strong RTP. Below are the proposed modes of interactions of B[f]Q with silica gel without binder and silica gel with binder.

Silica gel without binder

It is generally accepted that adsorption of compounds on silica gel occurs via hydrogen bonding between the adsorbate and the silanol groups of the silica surface. Snyder (16,17) indicated that generally aromatic compounds are adsorbed flatly on silica gel (i.e., parallel to the surface experienced by the adsorbate). It seems that only a flatwise adsorption mechanism would afford the compound the rigidity necessary for RTP. Therefore, any discussion of an adsorption mechanism which follows assumes flatewise adsorption of the adsorbate on the solid surface. Because weak RTP was observed from B[f]Q spotted from 0.1 M HCl ethanol solution on MN silica gel without organic binder, it seems reasonable to conclude that the RTP observed in this case is a result of hydrogen bonding between $B[f]QH^+$ and the silanol groups.

The acid serves to protonate B[f]Q, but because it must be added in excess of the stoichiometric amount of B[f]Q to obtain a signal another role of the acid is suggested. Deanin (18) discussed the Si-O-Si bond and its lability to acids. It therefore seems possible that the excess acid would attack siloxane groups to produce more silanol groups which would then be available for hydrogen bonding with the π electrons of the nitrogen heterocycles. Apparently, the hydrogen bonding between the silanol groups and $B[f]QH^+$ hold the molecules rigidly enough for weak RTP to occur.

Silica gel with binder

When acidic polymers or their salts, such as polyacrylic acid, are used as binders for commercial silica gel chromatoplates, another adsorbate-solid surface interaction is implicated. The results of the acid studies,

the luminescence, reflectance, infrared, and binder studies clearly indicated that enhanced RTP signals were only obtained when the binder was in its acidic form. Furthermore, the presence of many carboxyl groups dispersed throughout the silica established sites for strong hydrogen bonding interaction. For EM chromatoplates, polyacrylic acid is present in the range of 0.1-10% (19). Infrared results with mixtures of polyacrylic acid and silica gel without binder indicated the amount of binder at about 5% by weight.

Any reasonable proposed mechanism of interaction between $B[f]QH^+$ and EM silica chromatoplates, which contain an acidic polymer as its binder, must account for possible interactions between the adsorbed compound and the binder. The most logical adsorbate-solid surface interaction would be to propose hydrogen bonding between the π electron systems of the adsorbate and the carboxyl groups of the binder. Since the acidic polymer is a stronger acid than the silanol group, the carboxyl groups would be expected to form stronger hydrogen bonds with the π ring system of $B[f]QH^+$ and therefore hold the compound more rigidly to the surface. For these reasons, it appears $B[f]QH^+$ exhibited stronger RTP adsorbed on polyacid-NaCl mixtures than on pure silica gel. Because the reflectance data and fluorescence data indicated $B[f]QH^+$ was adsorbed on the chromatoplate in the protonated form, it seems probably that the $\rangle NH^+$ entity could form hydrogen bonds with the carbonyl oxygen of the carboxyl group.

As additional evidence for the hydrogen bonding mechanism, the hydrocarbon analog of B[f]Q, phenanthrene, was placed on a 5% polyacrylic acid-NaCl mixture from ethanol solution. After drying, the phenanthrene exhibited a strong RTP. Phenanthrene also exhibited a moderate RTP on EM silica gel chromatoplates when spotted from 0.1 M HCl ethanol solution. These results

suggested the excess acid converted carboxylate groups to carboxyl groups which then interact strongly with phenanthrene. Because phenanthrene has no heteroatom, it seems reasonable to conclude the main interaction responsible for the enhanced RTP observed from this coumpound is hydrogen bonding between the carboxyl groups of the binder and the π electron system of phenanthrene.

The separation of benzo[f]quinoline and phenanthridine from shale oil and identification of RTP

A method for the separation of B[f]Q and phenanthridine (PH) in shale oil employing a combination of column chromatography and high performance liquid chromatography (HPLC) was developed. The RTP spectra of the compounds adsorbed on a dried acid treated section of a silica gel chromatoplate was used for identification. Experimental details are given at the end of this section.

The initial separation step involved a chromatographic open-column aluminum oxide procedure that was a modification of a method developed by Schiller and Mathiason (20). Next, the fraction containing B[f]Q and PH was injected onto a HPLC μBondapak C_{18} column and a methanol:water (50:50 $^{v}/_{v}$) mobile phase was used to separate the fraction. The components corresponding to the B[f]Q and PH chromatographic peaks were collected, transferred to chloroform and then injected onto a HPLC μPorasil n-heptane:isopropanol (99.5:0.5 $^{v}/_{v}$) chromatographic system. The chromatographic peaks that corresponded to B[f]Q and PH were collected separately. The separate solutions were spotted on previously acid treated silica gel chromatoplate sections; the chromatoplate section were dried; and the RTP excitation and

emission spectra recorded. The RTP spectra for the suspect components matched the RTP spectra of the corresponding standards. With the retention time data from HPLC and the excellent match with the RTP spectra, it was concluded that B[f]Q and PH were isolated and identified. The RTP approach was not applied further because the work was done near the end of the contract period. The RTP has substantial potential to be coupled with HPLC as shown by this work. Small samples can be handled readily and in addition to RTP, fluorescence data can be obtained on many compounds. Thus from a component isolated by HPLC the following information readily can be obtained for luminescent compounds: fluorescence excitation (or phosphorescence) spectrum, fluorescence emission spectrum, phosphorescence emission spectrum, and phosphorescent lifetime of some compounds. This is a wealth of information that can be used to characterize and identify components in shale oil and other energy-related samples and should be developed further.

EXPERIMENTAL

Apparatus

Glass columns, 500mm X 11mm i.d., were employed for the column chromatography steps. The liquid chromatograph used was a Waters model ALC/GPC244 equipped with a model 6000-A pump, a U6K injector, a free standing ultraviolet detector set at 254nm, and 10mv strip chart recorder. The HPLC columns were 3.9mm i.d. X 30cm columns prepacked and obtained from Waters Associates, Milford Mass. The columns used were a μPorasil and a μBondapak C_{18}. A Waters SEP-PAK C_{18} cartridge was employed to place a sample initially in methanol water into chloroform prior to injection onto the μPorasil column. RTP excitation and emission spectra

were obtained with a Perkin-Elmer MPF-2A fluorescence spectrophotometer equipped with a phosphoroscope accessory. A 150-W Xe arc lamp and a 1P28 photomultiplier tube were employed.

Reagents

Methanol, n-heptane, and chloroform were HPLC grade. The methanol and chloroform were obtained from J.T. Baker, and the n-heptane was obtained from Fischer Scientific or Burdick and Jackson Laboratories, Inc. Distilled water was filtered through a Millipore type - G-S 0.22μm filter and methanol, n-heptane and chloroform were filtered through a Millipore type F-H ~0.5μm filter. The isopropanol was Mallinckrodt SpectrAR-grade and was used without filtering. The n-hexane and toluene were reagent grade and were used without further purification. Absolute ethanol was distilled. B[f]Q and PH standards were obtained from commercial sources and were recrystallized from ethanol and distilled water. The alumina used for the column chromatography was Brockmann activity I. The silica gel chromatoplates employed for the RTP work were aluminum backed from EM Laboratories, Elmsford, N.Y.

Procedures

Alumina Column Chromatography. The column chromatography steps employed were a modification of a procedure developed by Schiller and Mathiason (20) for coal derived solids and heavy-liquids. An accurately weighed sample of shale oil, ~0.05 g, was dissolved in about 2 ml of chloroform. Two grams of neutral aluminum oxide were added to the sample solution. The alumina containing the sample was in a small evaporating flask and was placed on a Buchi rotary evaporator so the chloroform could be removed under vacuum.

The flask was kept at about 30°C by a constant temperature water bath. The dried alumina containing the shale oil sample was then placed on top of a glass column containing about 3-g of alumina. The interface of the 2 g of alumina with the adsorbent will be referred to as the original sample-adsorbent interface. The sample was eluted with 20 ml of n-hexane, followed by a 50 ml toluene elution. Standards were employed to determine that B[f]Q and phenanthridine were located in a band just under the original sample-adsorbent interface following the toluene elution. Therefore, the adsorbent was removed from the glass column and a dark band just under the original sample-adsorbent interface, which contained B[f]Q and phenanthridine, was cut from the adsorbent and placed in an evaporating flask. The 2 cm section cut from the column adsorbent was dried under vacuum in a constant temperature water bath of 30°C. The dried sample was placed in another glass column on top of 3 g of alumina and was eluted with chloroform. Only one dark band migrated and this entire band was collected in an evaporating flask by collecting about 30-40 ml of chloroform from the column. This sample was evaporated just to the point of dryness, and the ethanol soluble portion was dissolved in ethanol and quantitatively transferred to a 1 ml volumetric with 3 ethanol washes. The sample containing B[f]Q and phenanthridine was then ready for further separation steps via HPLC.

High Performance Liquid Chromatography C_{18} Separation. The HPLC separation procedures described below were a modified version of a procedure for the separation of aza-arenes reported by Dong and Locke (21). A 30 µL portion of the ethanol soluble fraction collected from the alumina column steps described above was injected onto a µBondapack C_{18} column which had

been equilibrated with a methaol-water (1:1 v/v) mixture at a flow rate of 1.3 mL/min. Standards of B[f]Q and phenanthridine were run to determine their retention times. The samples from the column which corresponded to the elution of B[f]Q and phenanthridine from the column were collected into one collecting tube. Following sample collection the C_{18} column was purged with methanol until a reasonable baseline was established.

Sample Preparation Using a C_{18} SEP-PAK. Sample preparation was required prior to separation on μPorasil because injecting a water sample onto a μPorasil column can change the activity of the column. It was necessary to place the sample which was collected from the C_{18}HPLC column in methanol:H_2O into chloroform so injection onto a μPorasil column would have no deleterious effects on the column. The methanol:water sample solution was evaporated to 1-2 mL by forcing N_2 over the solution. This solution was placed on a C_{18} SEP-PAK mini-column with the aid of a slight vacuum. The sample adsorbed on the SEP-PAK column was eluted into a collecting tube with 3 ml of chloroform.

High Performance Liquid Chromatography μPorasil Separation. The chloroform sample collected from the SEP-PAK column was concentrated to about 30 μL by blowing N_2 over the solution. Care was taken to avoid taking the sample to complete dryness, because both B[f]Q and phenanthridine were found to be volatile and could be lost in this manner. The entire chloroform sample solution was injected onto a μPorasil column which had equilibrated with n-heptane:isopropanol (99.5:0.5 ^{v}/v) at a flow rate of 1.5 ml/min. Peaks of the chromatogram which corresponded to the elution of B[f]Q and phenanthridine from the μPorasil column were collected in separate collecting tubes.

Room Temperature Phosphorescence Identification. The solutions collected from the μPorasil column containing the B[f]Q and phenanthridine suspect compounds from a shale oil sample were concentrated to about 10 μL with dry N_2, again taking care not to take the sample to complete dryness. To the concentrated sample solutions one drop of distilled ethanol was added. The solutions were spotted with a 10 μL Hamilton syringe on separate sections of an EM silica gel chromatoplate which had both been spotted with 3 μL of a 0.1 M HCl ethanol solution. After spotting, the chromatoplate sections were placed in an oven and allowed to dry for 10 min at 105-110°C, removed from the oven and allowed to cool to room temperature before the RTP excitation and emission spectra were recorded with the fluorescence spectrophotometer.

E. PUBLICATIONS RESULTING FROM THIS WORK

1. R.J. Hurtubise, J.D. Phillip, and G.T. Skar, "Determination of Benzo[a]pyrene in a Filtered Retort Water Sample by Solid-Surface Fluorescence," Anal. Chim. Acta., 101, 333(1978).

2. R.J. Hurtubise and J.D. Phillip, "Characterization of Polycyclic Aromatic Hydrocarbons in Shale Oil by Chromatography and Fluorescence Densitometry," Anal. Chim. Acta, 110, 245(1979).

3. C.D. Ford and R.J. Hurtubise, "Design of a Phosphoroscope and the Examination of Room Temperature Phosphorescence of Nitrogen Heterocycles," Anal. Chem., 51, 659(1979) (partial support).

4. C.D. Ford and R.J. Hurtubise, "Room Temperature Phosphorescence of Nitrogen Heterocycles Adsorbed on Silica Gel," Anal. Chem., in press. (partial support).

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