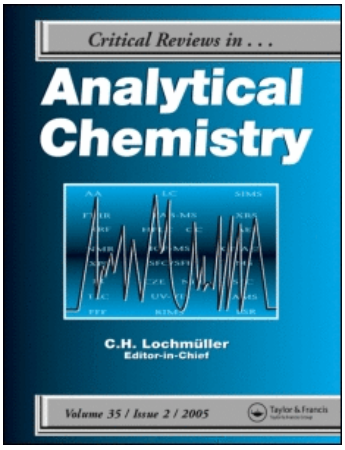

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Spectroscopic Methods of Analysis for Polycyclic Aromatic Hydrocarbons in the Aqueous Environment

David J. Futoma; S. Ruven Smith; John Tanaka; Peter C. Uden

To cite this Article Futoma, David J. , Smith, S. Ruven , Tanaka, John and Uden, Peter C.(1982) 'Spectroscopic Methods of Analysis for Polycyclic Aromatic Hydrocarbons in the Aqueous Environment', Critical Reviews in Analytical Chemistry, 13: 2, 117 — 154
To link to this Article: DOI: 10.1080/10408348208542751
URL: http://dx.doi.org/10.1080/10408348208542751

SPECTROSCOPIC METHODS OF ANALYSIS FOR POLYCYCLIC AROMATIC HYDROCARBONS IN THE AQUEOUS ENVIRONMENT

Authors: **David J. Futoma**
Department of Chemistry
University of Connecticut
Storrs, Connecticut

S. Ruven Smith
John Tanaka
Department of Chemistry and
Institute of Materials Science
University of Connecticut
Storrs, Connecticut

Referee: Peter C. Uden
Department of Chemistry
University of Massachusetts
Amherst, Massachusetts

TABLE OF CONTENTS

I. INTRODUCTION

A number of new techniques based on spectroscopic principles are being developed for the analysis of PAH as possible alternatives to the chromatographic methods discussed in our earlier review.[1] The methods discussed in the following sections address themselves to one or both of two broad developmental goals. The first is the need for analytical procedures which are (1) selective for several PAH, (2) capable of detecting subnanogram amounts of PAH, (3) able to provide results on a real-time basis, (4) portable, inexpensive, and easy enough to be automated or run by technicians, all without chromatographic preseparations. Synchronous spectroscopy, room temperature phosphorimetry, and matrix isolation spectroscopy are all techniques which can satisfy these requirements. The second important goal in developing analytical instrumentation is to devise detectors which are capable of obtaining more information especially from HPLC and less urgently from GC instruments. At present, the mass spectrometer is the only detector for HPLC and GC capable of providing qualitative identifications of PAH. For this reason, matrix isolation fluorescence and infrared spectroscopy, low temperature Shpol'skii fluorescence spectroscopy, opto-electronic image detectors, and wavelength modulation and derivative spectroscopy are all being developed to provide more sophisticated information on HPLC and GC eluants.

II. BASIC SPECTROSCOPIC PROPERTIES OF PAH

Polycyclic aromatic hydrocarbons are a class of compounds built around the simplest member, naphthalene. The large family of compounds is formed by the addition of as many as five to eight benzene rings to naphthalene, and a large number of isomeric species are generated by virtue of the different sites where the benzene rings can fuse. The family of these compounds is called PAH.

Before examining the new and sophisticated spectroscopic analytical techniques, we will review the basic ultraviolet absorption and fluorescence properties of PAH and, in addition, the simple ultraviolet and fluorescence detector which is routinely used in most modern high pressure liquid chromatographs. An examination of the shortcomings of these detectors in terms of both selectivity and sensitivity towards PAH will make obvious the need for further instrumental development.

PAH can usually be isolated as a class of compounds from other organics found in the environment. However, within the family of PAH, differences in spectral appearance are sometimes very slight and undetectable, e.g., ultraviolet absorption and fluorescence spectra of PAH in solution at room temperature are quite similar. First, the absorption and emission spectra range over wavelength intervals of as much as 200 to 400 nm, a common feature of such spectra in solution at room temperature caused by the superposition of vibrational and rotational contributions on the electronic spectra. Second, the structures of many isomeric PAH are naturally quite similar and this gives rise to nearly identical spectra. Although fluorescence emission spectra could theoretically be generated without interference from other PAH by selectively exciting at an excitation wavelength unique to one particular PAH, this is difficult to accomplish experimentally since some other PAH will almost always exhibit some absorbance in the usual range of excitation wavelengths, 200 to 600 nm. This certainty arises to a large extent because of the broad-banded nature of the excitation spectra. Tables and fluorescence spectra of many PAH along with physical and chemical spectroscopic information are listed in Reference 2.

The first spectroscopic detectors for PAH were very simple instruments with the resulting limitations. The simplest detector was based on the $\pi - \pi^*$ absorptions exhib-

ited by the aromatic ringed PAH. Detectors with only one accessible source wavelength (usually 254 nm to coincide with the strong 254-nm line emitted by mercury arc sources) and continuously variable wavelength detectors with xenon arc or tungsten halogen continuum sources were employed. Fluorescence detectors were the next step in the development of PAH detectors since essentially every PAH exhibits strong ultraviolet and visible fluorescence emission. These detectors added selectivity since selective excitation could theoretically be used and because other non-PAH organics were non-fluorescent and could be left undetected.

The above discussion makes it obvious that there are a number of shortcomings in presently used detectors. First, unless complete chromatographic resolution is possible, interference in the absorption and emission spectra will occur from other organics and PAH giving rise to analytical errors. If the broad-branded spectra could be reduced to narrow-lined spectra, more than one component in a chromatographic fraction could be identified, and if narrow-lined excitation spectra were present, unique wavelengths for selective excitation might be possible. Another goal is to reduce line width so that characteristic fingerprint spectra can be obtained. This goal is the basis of the low temperature fluorescence studies to be discussed. A second way of overcoming the problem of interfering spectra is to modify the instrumental detector through addition of derivative, wavelength modulation and other computer-based spectra manipulation capabilities. To improve the sensitivity that can be obtained from continuum sources, laser sources are being developed.

III. ROOM TEMPERATURE PHOSPHORIMETRY

The phenomenon of room temperature phosphorescence (RTP) has attracted considerable attention as the basis for a rapid and sensitive analytical technique for determination of PAH.[3-11] Parker et al.[11] have thoroughly reviewed the development of RTP into an analytical technique with focus on the physical aspects of the phenomenon.

Normally, the measurement of phosphorescence is possible only at cryogenic temperatures such as 77 K, where the solute is locked into the solvent matrix to prevent collisional deactivation. However, at room temperature the solute can achieve this same rigidity by adsorption onto the surface of a filter paper support which provides a similar matrix to that observed for a solution frozen at 77 K.

There are four basic stages in an RTP analysis and these are illustrated in Figure 1. Each of these various procedures have been examined in-depth and reported in the references.[3,4,11] The initial step is to find a suitable substrate for the analysis. Filter paper is generally found to give the optimum response, in terms of signal-to-noise ratio. Adsorption and hydrogen bonding effects have been cited[11] as the most probable cause for the rigid environment experienced by ionic- and hydroxyl-bearing organic solutes. The situation for PAH is somewhat more complex. Adsorption would not be expected between a nonpolar compound and a polar adsorbent such as paper. However, the addition of a heavy atom is also necessary to enhance an otherwise weak phosphorescence signal. The formation of π-complexes is generally considered to be one of several factors having a bearing on the PAH-substrate interaction.[11] This effect is discussed in the next paragraph. Schleicher and Schuell 604 and 2040A appear to be the preferred brands of filter paper.[3,10] Various filter paper pretreatments, e.g., moistening with 1 *M* sodium acetate, citrate, and malonate followed by drying, gave rise to poorer signal-to-noise ratios and were therefore avoided.[4] As suggested in Figure 1, a continuously feeding paper roll could be designed to provide for an automatic analytical system.

The sample of PAH in solution in most cases requires the addition of the salt of a heavy atom to enhance the phosphorescence intensity. The addition of the heavy atom is

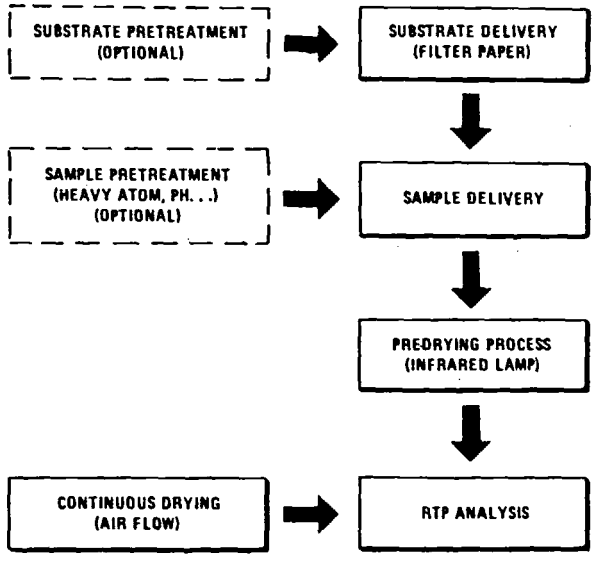

FIGURE 1. Block diagram of the room temperature phosphorescence analytical scheme.

believed to increase the spin-orbit coupling necessary to permit otherwise forbidden spin-transitions to occur, e.g., the singlet-triplet intersystem crossing rate is enhanced thereby increasing phosphorescence at the expense of decreased fluorescence.

Bower and Winefordner[4] investigated the effects of a number of heavy atoms on the RTP intensity of PAH adsorbed on filter paper. They found the degree of enhancement to be greatest for thallium(I) ions. They suggested the possible formation of a π-complex between the metal ion and the PAH as the means by which structural rigidity and consequently the phosphorescence intensity increased.

Vo-Dinh and Hooyman[7] have investigated in an empirical manner the effect of heavy atoms on the RTP of PAH. For PAH concentrations of 10^{-3} M or below, the optimum concentration of heavy atom was found to be in the range of 0.5 to 2 M. Maximum enhancement of phosphorescence intensity was achieved under these conditions. To best ensure compatibility between the PAH and heavy-atom solution, both solvent systems were similar: ethanol and ethanol-water mixtures.

Vo-Dinh and Hooyman[7] tested 20 heavy atoms to determine their effect on the enhancement of RTP for several PAH. The six heavy-atoms salts which exhibited the best performance in this regard were silver nitrate ($AgNO_3$), cesium iodide (CsI), lithium perchlorate ($LiClO_4$), sodium bromide (NaBr), sodium iodide (NaI), and lead acetate ($Pb(OAc)_2$). The heavy-atom enhancement factors for these six salts when added to a number of PAH solutions are listed in Table 1. This factor f_s^{HA} is defined by the authors[7] to be the ratio of the phosphorescence signal with heavy atom (HA) to that without the heavy atom, taking into account the contribution of the background intensities of the filter paper and heavy-atom solution. Of particular note in Table 1 is the variations in enhancement factors for different heavy atoms and different PAH. Variations in enhancement factors for certain combinations of heavy atom and PAH can be exploited to preferentially increase the phosphorescence intensity of specific components in a mixture. Vo-Dinh and Hooyman[7] have applied this factor in analyses of multicomponent mixtures of PAH (see below).

The next step in the RTP procedure is the spotting of the sample onto the filter paper. This can be carried out in a straightforward manner using a micropipet or syringe capable

Table 1
PHOSPHORESCENCE ENHANCEMENT FACTOR f_s^{HA} FOR SEVERAL PAH

Compound	$AgNO_3$ 0.5 *M*	CsI 0.2 *M*	NaI 2 *M*	NaBr 2 *M*	$Pb(OAc)_2$ 0.5 *M*	$LiClO_4$ 2 *M*
Acridine	10	2	1.5	8	40	N[a]
Benzo(a)pyrene	N	24	27	30	240	9
Benzo(e)pyrene*	10	95	10	28	380	2
2,3-Benzofluorene	18	110	35	50	110	2
Carbazole	N	72	75	18	35	2
Chrysene	25	80	35	33	270	4
1,2,3,4-DBA*	150	580	130	110	750	10
1,2,5,6-DBA*	50	350	150	220	250	40
Dibenzocarbazole*	N	410	400	120	60	4
Fluoranthene	10	55	20	20	200	4
Fluorene	N	110	35	45	150	6
1-Naphthol	N	140	140	50	60	1.5
Phenanthrene	N	30	5	20	110	N
Pyrene	7	80	40	9	410	7
Quinoline	40	15	12	75	210	4

Note: Concentrations of all compounds were 10^{-4} *M* except the (*) compounds which were at concentrations 5×10^{-5} *M*. The dibenzocarbazole above is 13H-dibenzo(a,i)carbazole.

[a] N = no enhancement ($f_s^{HA} < 1$).

of delivering microliter volumes. Three microliters is an average sample size for both the PAH solution and the heavy-atom solution. The heavy-atom solution is usually spotted first, followed immediately by the PAH or analyte solution.

A predrying step immediately follows the sample introduction. The actual length of time and the conditions under which the drying occur are very important parameters. A 10-min predrying time was found to be optimum in terms of the resulting signal-to-noise ratio.[3-5] An infrared lamp was found to be more efficient for this procedure than the use of desiccators, blowers, or ovens.[6] The drying process must in some manner strengthen the bond between the PAH molecule-heavy atom pair and the filter paper. Parker et al.[11] reviewed the research efforts directed towards understanding the effects of moisture and oxygen on the RTP signal and were easily able to conclude that the presence of both can result in significantly depressed RTP intensity depending upon their concentrations. Bower and Winefordner[4] have noted that the RTP intensity of pyrene was 75% lower in air than in a nitrogen or argon atmosphere. A smaller decrease in RTP intensity was observed for more highly polar organics. In analyses of PAH it was concluded[4] that as a result of the weaker adsorption which might be expected between PAH and filter paper, oxygen quenching would be more effective in decreasing the RTP signal. Parker et al.[11] have noted that oxygen quenching appears to be more effective as the atmospheric humidity increases suggesting that the moisture helps to weaken the solute-substrate interaction allowing the oxygen to more easily quench the phosphorescence signal. The recommended conditions for analysis are thus an inert and dry atmosphere for RTP measurements. Dry nitrogen gas was chosen as a suitable atmosphere for the RTP determinations.[4]

The final step, the measurement of the room temperature phosphorescence, can be made on a spectrophotofluorimeter modified for the addition of a phosphoroscopic attachment.[3] The sample compartment must be altered to allow for samples spotted onto paper. By means of a rotating mirror assembly, the excitation light beam could be focused onto the surface of the filter paper which moves horizontally across the slit

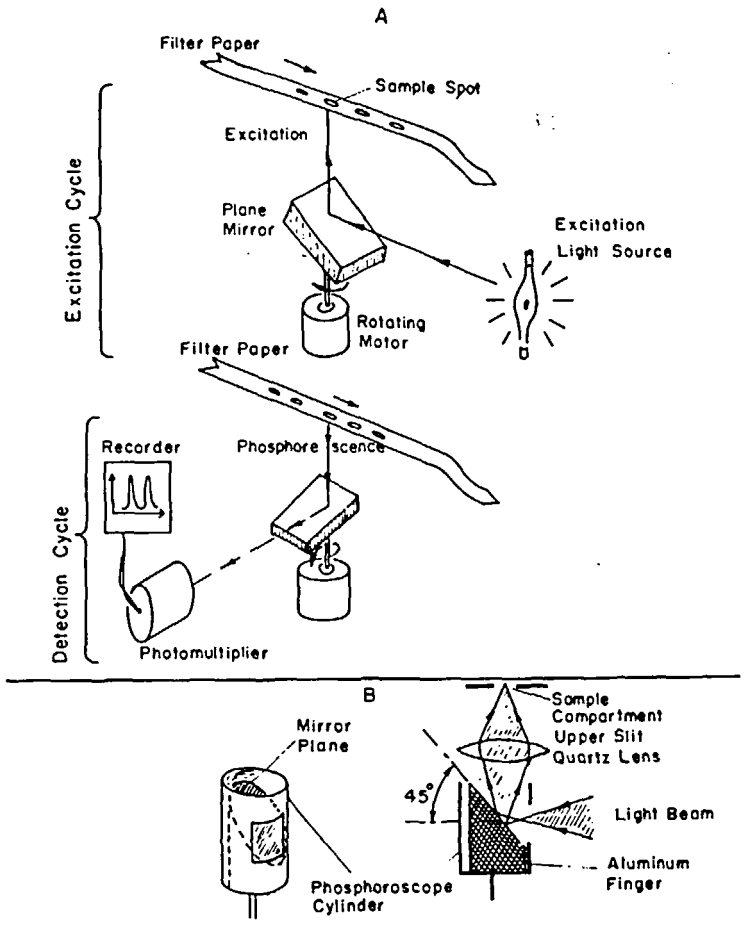

FIGURE 2. (A) Principle of phosphorimetric excitation and detection with the rotating mirror phosphorimeter. (B) Design of the rotating mirror assembly.

located at the top of the sample compartment. As the cylindrical reflecting surface is finally moved into the emission path, the phosphorescence can be reflected by the mirror into the detection system. The excitation and emission cycles are illustrated in Figure 2 along with the rotating mirror assembly.

The technique of RTP can be used in conjunction with synchronous scanning (discussed in the next section) or combined with first and second derivative capabilities (discussed in Section VII). Vo-Dinh and others[6-10] have applied RTP to a number of environmental and model samples containing PAH. Taking advantage of the selective enhancement of certain heavy atoms for particular PAH, Vo-Dinh and Hooyman[7] were able to analyze a seven-component mixture of PAH, their results are listed in Table 2. They also determined the limits of detection for several PAH after finding the optimum heavy atom-PAH pair, and as seen in Table 3, nanogram and subnanogram levels are detectable. Vo-Dinh et al.[10] have also used this selective heavy-atom technique to determine the trace concentrations of several PAH in Synthoil (a coal liquid from a hydrodesulfurization process). Benzo(a)pyrene, chrysene, and other potentially carcinogenic PAH were characterized at concentrations ranging from tens to thousands of parts per million. A RTP analysis with the synchronous scanning mode is discussed in the next section.

Table 2
QUANTITATIVE RTP ANALYSIS OF A SEVEN-COMPONENT MIXTURE OF PAH

Compound	Heavy atom used	λ_{ex} (nm)	λ_{em} (nm)	Actual amount (ng)	Amount determined (ng)
Benzo(a)pyrene	$Pb(OAc)_2$	395	688	75	80 ± 9
Benzo(e)pyrene	$Pb(OAc)_2$	335	543	7.5	6.8 ± 0.7
Chrysene	NaBr	330	518	7	6.5 ± 0.6
1,2,3,4-DBA	CsI	295	567	50	53 ± 5
Fluorene	NaBr	270	428	48	43 ± 4
Phenanthrene	NaBr	295	474	27	26 ± 3
Pyrene	$Pb(OAc)_2$	343	595	6	5.6 ± 0.6

Table 3
LIMITS OF OPTICAL DETECTION (LOD) OF SEVERAL PAH BY RTP ANALYSIS

Compound	λ_{ex} (nm)	λ_{em} (nm)	Heavy atom	LOD (ng)
Acridine	360	640	$Pb(OAc)_2$	0.4
Benzo(a)pyrene	395	698	$Pb(OAc)_2$	0.5
Benzo(e)pyrene	335	543	CsI	0.01
2,3-Benzofluorene	343	505	NaI	0.028
Carbazole	296	415	CsI	0.005
Chrysene	330	518	NaI	0.03
1,2,3,4-DBA	295	567	CsI	0.08
1,2,5,6-DBA	305	555	NaI	0.005
Dibenzocarbazole[a]	295	475	NaI	0.002
Fluoranthene	365	545	$Pb(OAc)_2$	0.05
Fluorene	270	428	CsI	0.2
1-Naphthol	310	530	NaI	0.03
Phenanthrene	295	474	NaBr	0.007
Pyrene	343	595	$Pb(OAc)_2$	0.1

[a] 13H-dibenzo(a,i)carbazole.

Chromatographic methods will never be replaced as a technique for providing complete analysis of samples containing hundreds to thousands of components. However, RTP is quite suitable as a method for routine screening of large numbers of samples due to its simplicity. It does not require cryogenic apparatus and utilizes only inexpensive filter paper as a substrate. Even unfractionated samples such as Synthoil can be monitored directly to provide rapid results for a particular PAH concentration. More detailed RTP analyses can be run on mixtures of 5 to 10 components at the cost of a small increase in analysis time. The RTP technique should be considered by all laboratories for the particular merits it possesses.

IV. SYNCHRONOUS LUMINESCENCE SPECTROSCOPY

In conventional fluorescence or phosphorescence spectroscopy, an emission spectrum is generated by scanning the emission wavelength, λ_m, as the sample is irradiated at a single excitation wavelength, λ_x. Analogously, an excitation spectrum results from

scanning the excitation wavelength while recording the emission signal at a single wavelength. The third possibility involves varying simultaneously λ_x and λ_m while keeping a constant wavelength interval $\Delta\lambda$ between them. The resulting signal trace, a "synchronous spectrum", differs markedly from simple excitation and emission spectra. The synchronous luminescence intensity depends on the character of the normal excitation and emission spectrum as well as the wavelength difference, $\Delta\lambda$, between the excitation and emission wavelength. The intensity can be expressed as

$$I_s = KE_x(\lambda_m - \Delta\lambda)E_m(\lambda_m)cd \qquad (1)$$

where E_x is the excitation spectrum at a wavelength of $(\lambda_m - \Delta\lambda)$, E_m is the normal emission spectrum signal at λ_m, c is the analyte concentration, d is the sample cell thickness, and K is a characteristic luminescence constant. A more complete treatment of the above can be found in References 12 and 13.

Figure 3 illustrates the difference in physical appearance between the normal excitation and emission spectra for PAH and the resulting synchronous spectra for solutions of anthracene, phenanthrene, and perylene. The fluorescence excitation and emission spectra of perylene span an interval from 350 to 550 nm while the synchronous signal shows one peak centered at 440 nm with a bandwidth of approximately 20 nm. This spectral simplification arises as a result of the overlap of two spectral patterns, the emission and excitation spectra, which are essentially mirror symmetric. The excitation spectrum is limited on the long wavelength end and the emission spectrum on the short wavelength region, thus their product will be necessarily limited in width.

The only requirement necessary to observe a narrow peak in the synchronous mode is that either the excitation or emission spectra exhibit resolved structure in a given spectral range. Only when both spectra are featureless will there not be a resolved synchronous signal. The most intense and narrow peak will result when the $\Delta\lambda$ parameter is chosen to equal the wavelength interval between the maxima in the excitation and emission spectra. The $\Delta\lambda$ parameter will thereby depend on the individual characteristics of the PAH excitation and emission spectra. A detailed treatment of the effect of this parameter on the nature of the synchronous spectrum can be found in Reference 12. The important results for the analyst interested in PAH are the following: (1) multicomponent mixtures of PAH can be more readily analyzed from the resulting simplified spectra and (2) the $\Delta\lambda$ parameter can enable the "selective" monitoring of one PAH in the presence of others if $\Delta\lambda$ is unique to one PAH. An example of the spectral simplification is shown in Figure 4. The top half of the figure is the conventional fluorescence emission spectrum of a mixture of five PAH where it is quite clear that identification of individual PAH is nearly impossible. The resulting synchronous spectrum shows five peaks each of which can be correlated with an individual PAH.

Vo-Dinh[12] has discussed the possibility of correlating the synchronous signal with the structure of the PAH compound. In general, it is noted that the spectrum of a higher ring-number cyclic compound occurs at a longer wavelength than the spectrum of a lower ring-number compound. A data chart with theoretical predictions of the synchronous signal for particular PAH was constructed and can be found in Reference 12. The bands show excellent fit to experimental data according to Vo-Dinh.

The technique has been developed for rapid screening of aqueous pollutants and has been applied to monitor naphthalene and its methyl derivatives in the byproduct water of the synthane gasifier.[6] 1-Methylnaphthalene and 2-methylnaphthalene could be measured in the presence of one another with 1-ppb detection limits. Synchronous fluorescence spectroscopy was also used to analyze coal-gasifier waste water for its cresol and phenol content and all three cresol isomers could be distinguished.[6,14,15]

It is possible to apply the synchronous technique to RTP studies to add another degree

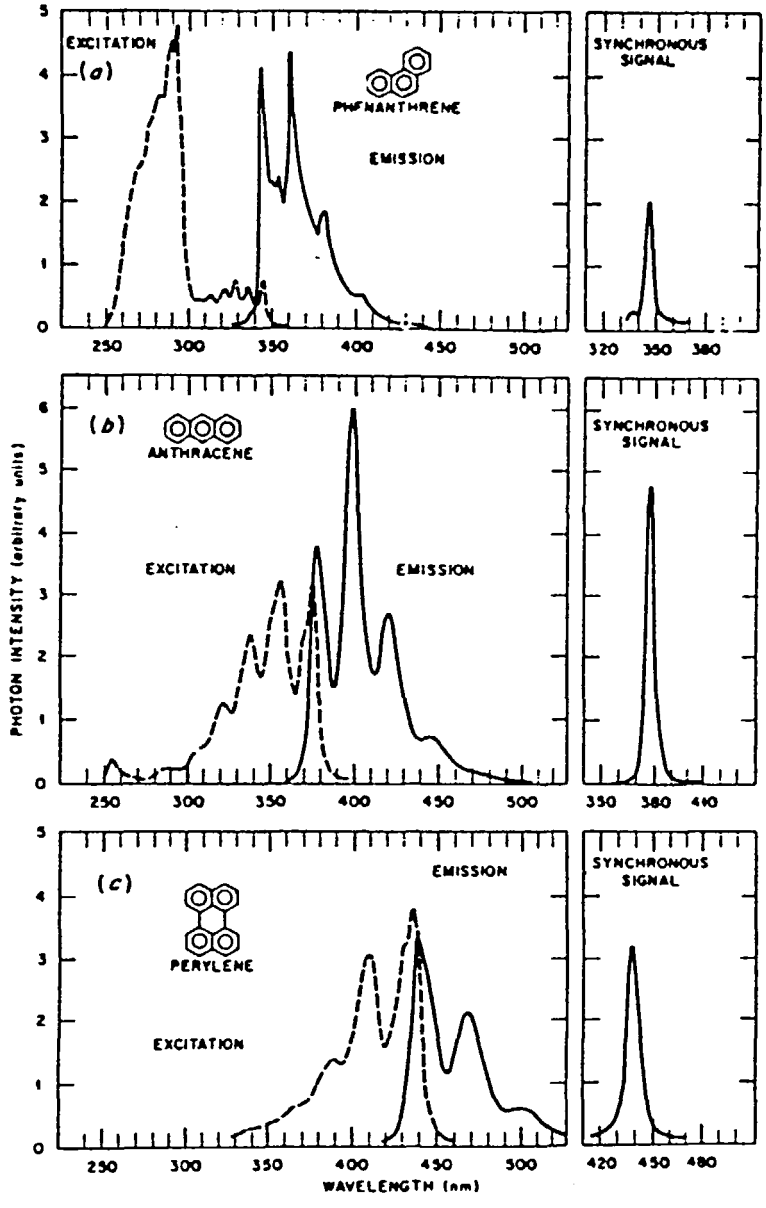

FIGURE 3. (a) Fluorescence excitation, emission, and synchronous spectra of phenanthrene. (b) Fluorescence excitation, emission, and synchronous spectra of anthracene. (c) Fluorescence excitation, emission, and synchronous spectra of perylene.

of selectivity. This added factor of selectivity is based upon the singlet-triplet energy difference for the PAH. This energy difference denoted as Δ_{st} is usually unique for a particular species. The phosphorimetric approach is exactly the same as that described earlier in the section on RTP analysis except for the conversion of the excitation and emission monochromators to the synchronous mode. Using the synchronous technique along with the unique Δ_{st} values for each PAH, several isomeric PAH can be quantitatively measured in the presence of each other.[16,17]

The basic approach is to determine optimum Δ_{st} values for each PAH and to set the

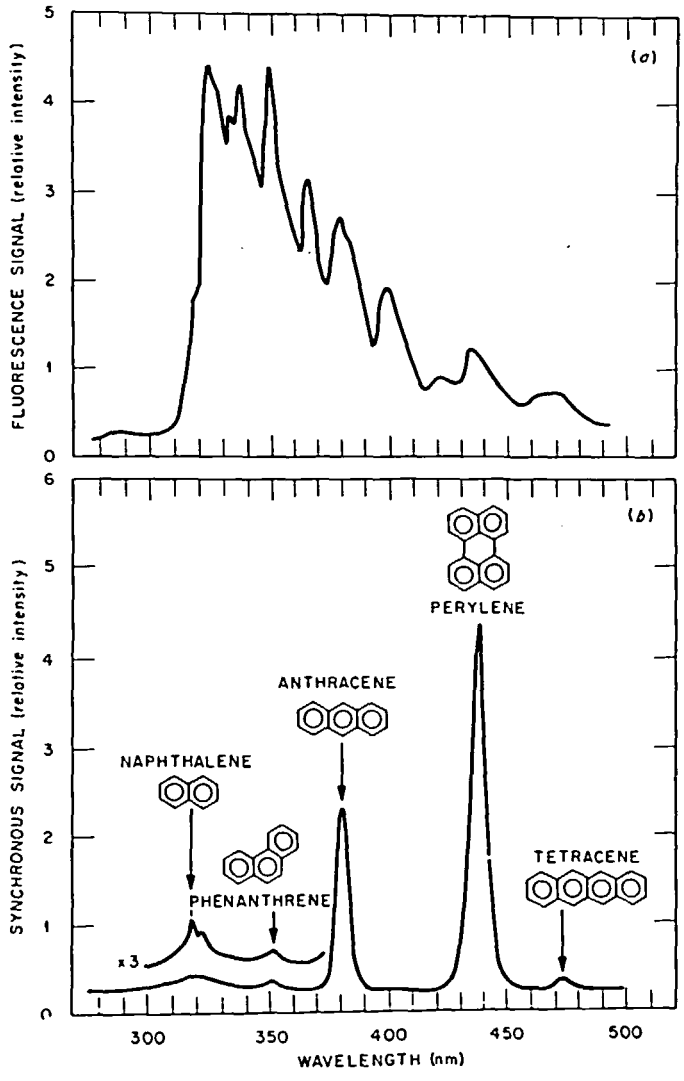

FIGURE 4. (a) Fluorescence spectrum of a mixture of naphthalene, phenanthrene, anthracene, perylene, and tetracene ($\lambda_{exc.}$ = 258 nm). (b) Synchronous signal ($\Delta\lambda$ = 3 nm) of the same mixture.

wavelength interval $\Delta\lambda$ in the synchronous mode equal to this singlet-triplet wavelength difference. An example of this involves an analysis of a mixture of 1,2,3,4- and 1,2,5,6-dibenzanthracene (DBA) where the energy differences Δ_{st} were found to be 274 and 252 nm, respectively.[14] In a sample of 42 ng of 1,2,3,4-DBA and 4.2 ng of 1,2,5,6-DBA, the synchronous RTP spectra were clearly differentiated and thus could be quantified as shown in Figure 5. This fact is important since 1,2,5,6-DBA is noted to be more carcinogenic than its isomer. Other isomeric PAH amenable to this synchronous RTP approach include the benzopyrenes. Table 4 lists the optimum Δ_{st} values for a number of PAH.

The synchronous RTP approach has been used by Vo-Dinh et al.[9,10,14,16] to analyze various environmental samples for individual PAH. Synthoil samples were analyzed for pyrene and fluorene after the synchronous mode narrowed the original RTP peak widths.

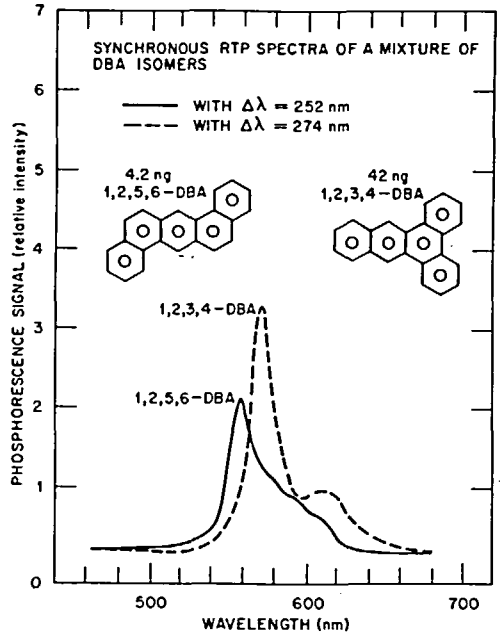

FIGURE 5. Synchronous RTP spectra of a sample containing 4.2 ng of 1,2,5,6-dibenzanthracene (DBA) and 42 ng of 1,2,3,4-DBA (see text).

Table 4
RTP EXCITATION AND EMISSION BANDS AND EXPERIMENTAL Δλ VALUES FOR SEVERAL PAH

Compound	Excitation peaks (nm)	Emission peaks (nm)	Optimal[a] Δλ values (nm)
Acridine	360	640	280
Chrysene	325	515	190
Fluorene	305	430	125
Naphthalene	275	472	197
Phenanthrene	250	460	210
Pyrene	350	600	250
Quinoline	315	465	150
Benzo(e)pyrene	335	543	208
Benzo(a)pyrene	392	690	298
1,2,5,6-DBA	301	555	254
1,2,3,4-DBA	296	570	274

[a] As determined experimentally.

Latz et al.[18,19] have recently cautioned against the sole use of synchronous spectroscopy for quantitative measurements on multicomponent samples. Noting that synchronous spectroscopy is an excellent fingerprinting tool, they point out that difficulties can arise in quantitative analyses which must be considered. One problem is the loss in spectral

detail arising from the use of the synchronous mode. Inconclusive quantitative results can arise from weak fluorescence signals, accidental wavelength coincidence, or masking and quenching effects. They note that effects like this occur also in conventional fluorimetry, but stand out more noticeably when excitation and emission spectra are measured and are more easily accounted for. Instrumental requirements are essentially identical to those for conventional fluorimetric analyses and as a result synchronous spectroscopy can be available to most laboratories. We believe that synchronous spectroscopy has great utility as a qualitative and even semiquantitative method, but until further results on quantitative measurements are available, other techniques, e.g., chromatographic, should be employed for total quantitative analyses.

V. SHPOL'SKII EFFECT LOW TEMPERATURE FLUORESCENCE SPECTROSCOPY

The Shpol'skii effect is observed if an aromatic solute, e.g., a PAH, is dissolved in a particular n-alkane solvent, frozen at 77 K or below, and excited at a wavelength capable of producing a luminescence spectrum. The observation made by Shpol'skii et al.[20] in 1952 which led to analytical applications was that this luminescence spectrum exhibited an extremely well-resolved fine-line structure characteristically unique for each solute. The postulated reason[21] for the fine structure is that in the rigid matrix the solute molecules which are held in strictly oriented sites and at the low concentrations are isolated from each other by large distances preventing mutual interaction. Unlike the case of solvents which forms transparent glasses at 77 K, where the glass fails to exhibit a short-range order[21] causing the electronic transitions to vary markedly with changes in the molecular field, the solutions of PAH in n-alkane solvents behave differently. The PAH solute molecules experience a well-defined molecular field which gives rise to the sharp-line electronic spectra.

The unique feature of the Shpol'skii effect is that the n-alkane solvent used to dissolve the PAH must be specifically matched to the solute. The length of the n-alkane must be of the same molecular dimension as the PAH molecule. The degree of complexity and uniqueness of the resulting luminescence spectrum depends upon this match. A comparison of the emission spectrum of anthracene in n-hexane and n-octane is shown in Figure 6, where it can be seen that a well-resolved fine-line spectrum is observed only in n-hexane. The solvent effect for 3,4,8,9-dibenzopyrene is also illustrated in Figure 6. The emission spectra of 20 μg/mℓ solutions of coronene in dioxane, pentanol, carbon tetrachloride, chloroform, bromoform, diethylether, dimethylformamide, and tetrahydrofuran were examined at 77 K. A Shpol'skii effect spectrum resulted only from a tetrahydrofuran solution. The addition of 10% cyclohexane to facilitate PAH dissolution had no effect upon the resulting spectra and was a common practice. More details on the solvent effect can be found in Reference 21.

Analytical studies of PAH by means of low temperature fluorescence utilizing the Shpol'skii effect have been widely published in the Russian literature of the late 1960s and early 1970s.[21] Qualitative and quantitative analyses of PAH in all types of environmental, foodstuff, and model samples were reported. Kirkbright et al.[21-23] published a three-part series of papers in *Analyst* reviewing most of the Russian literature pertaining to Shpol'skii effect studies on PAH. They critically examined the usefulness of the technique to future analytical applications. Their findings regarding the efficiency of various instrumental sample cells, light sources, and detectors are reviewed as well as the qualitative and quantitative results of recent investigators. A number of critical remarks in the literature on the limitations of the Shpol'skii effect are also reviewed, and a consensus outlook of the future of the technique is presented.

A spectrofluorimeter with a low temperature cell attachment is necessary for the

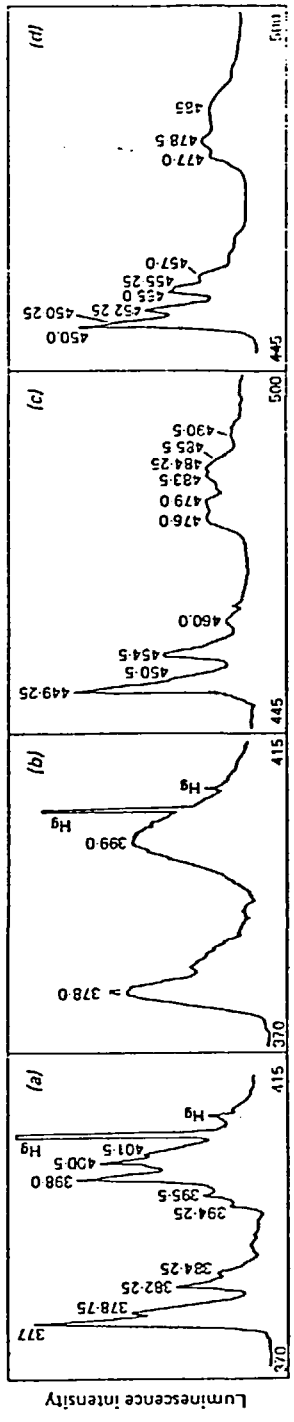

FIGURE 6. Solvent effect observed in the emission spectra at 77 K for anthracene with (a) n-hexane:cyclohexane solvent and (b) n-octane:cyclohexane solvent; and for 3,4,8,9-dibenzopyrene with (c) n-octane:cyclohexane solvent and (d) n-decane:cyclohexane solvent.

Table 5
DETECTION LIMITS OBTAINED AT 77 K WITH DIFFERENT EXCITATION SOURCES FOR SEVERAL PAH

Compound	λ_{em} (nm)	Detection limits (ppm)			
		150-W xenon-arc	Hg-vapor discharge lamp	He-Cd laser	
				325 nm	441 nm
Coronene[a]	445.05	2×10^{-3}[b]	1×10^{-3}[b]	5×10^{-4}	—
Benzo(a)pyrene[c]	403.00	2×10^{-4}[b]	1×10^{-4}[b]	1×10^{-4}	—
Benz(a)anthracene[c]	383.75	—	2×10^{-3}[b]	5×10^{-4}	—
Dibenz(a,h)anthracene[c]	394.25	—	5×10^{-3}[b]	3×10^{-3}	—
Perylene[a]	443.95	—	1×10^{-2}[d]	—	2×10^{-3}
Dibenzo(a,h)pyrene	449.15	—	1×10^{-4}[e]	—	7×10^{-4}

[a] In hexane.
[b] 300-nm interference filter.
[c] In octane.
[d] 250-nm interference filter.
[e] 300- or 325-nm interference filter.

analysis. Various excitation sources, sample cell assemblies, and detection systems were compared by Causey et al.[22] A 150-W xenon arc lamp continuum source, a medium pressure mercury vapor discharge lamp, and a 3-mW helium-cadmium laser were all employed as excitation sources. The laser's principal emission occurred at 325 or 441 nm which could be selected by the use of appropriate mirrors. The radiation from the xenon and mercury sources was selected by the use of interference filters with peak transmission wavelength of either 250 or 300 nm. The detection limits, expressed as the concentration of the compound studied that gave rise to an emission signal intensity-to-noise ratio of two, are shown in Table 5. The detection limits were generally lower by a factor of two when the mercury vapor lamp was used rather than the xenon-arc source. The mercury vapor lamp also gave rise to less scattered radiation from the sample at wavelengths near the measured emission wavelength. For compounds whose luminescence emission bands overlapped the mercury line radiation, the xenon-arc source is preferable. Causey et al.[22] concluded that both sources should be available for use in an analysis.

Preliminary studies with the helium-cadmium laser source indicated that even lower limits of detection were possible if the principal excitation maximum overlapped the narrow-line output from the source at 325 or 441 nm. In Table 5 little improvement in detection limits can be seen except for compounds which exhibited excitation maxima at the two laser lines. The need for a tunable dye laser source is pointed out by the authors.[22]

Causey et al.[22] compared a standard Dewar flask sampling system and a copper cryostat sample cell assembly. The Dewar system consisted of a silica sample tube (length 200 mm, i.d. 3 mm and wall thickness 0.6 to 1 mm). The sample tube (with samples of 0.3 to 0.5 mℓ) was immersed in liquid nitrogen contained in a Dewar flask with a transparent silica window that incident radiation can pass through in order to excite fluorescence in the sample which is then detected by the spectrofluorimeter. The advantages of the system were the rapid rate of cooling and the stable final temperature of 77 K. A number of disadvantages were present in this system, e.g., poor reproducibilities of emission intensities stemmed from difficulties in the positioning of the sample tube in the flask. In addition, problems resulted from the condensation of ambient water vapor on the external surface of the Dewar flask and within the liquid nitrogen. The signal

noise is elevated also by the constant boiling of the liquid nitrogen in the optical path. In an effort to eliminate these drawbacks, a new sample cell and cryostat assembly was constructed from copper. With the new assembly, the sample cell was isolated from the liquid nitrogen coolant and could actually be more reproducibly fixed in the excitation path. The rate of cooling was 8 to 10 times less (75 K/min vs. 720 K/min for a 0.6-mm thick silica cell) for this assembly, but the reproducibility of results was improved markedly. Relative standard deviations of 1.3 to 2.5% were obtained for measurements of emission intensity as opposed to values of 4 to 12% for the Dewar® flask system. Details on the two sample cell assemblies can be found in Reference 22.

Direct current integration, photon counting, and repetitive optical scanning used in conjunction with a signal averaging system were compared.[22] Improvements in detection limits were evident with the signal-integration technique, but the use of this method precluded the simultaneous qualitative analysis of all the PAH. The advantages of photon counting included improved precision in intensity results, but the technique did not lead to significantly lower limits of detection. In addition, there was considerable difficulty with the method as a result of the presence of high background light levels due to scattered source radiation. The use of repetitive optical scanning showed great potential for analyses at low sample concentrations of PAH due to increased analytical sensitivity. The application of the technique with signal averaging allowed maximum spectral resolution of the spectrofluorimeter at the very low light levels present when the slit widths were very narrow.

Qualitative analyses by Shpol'skii effect low temperature fluorescence spectroscopy have had widespread success, as witnessed by the large number of Russian papers.[21] Figure 7 shows the emission spectrum of a synthetic, eight-component PAH mixture.[21] It was found to be relatively easy to identify all eight components by identification of individual peaks. Numerous applications of the technique in qualitative analyses are present in the Russian literature and are reviewed in Reference 21.

Monarca et al.[24] have applied the method to determine the concentration of benzo(a)-pyrene in river, rain, and drinking water samples. A 125-W mercury lamp was employed with 325 and 375 nm excitation wavelengths available with the use of interference filters. A Dewar® flask sample cell assembly was used with silica tubes (3 mm i.d.) used as sample cells. Further instrumental details can be found in the original references.[21,24]

The water samples were subjected to liquid-liquid extraction with cyclohexane. The cyclohexane extract was dried and subsequently evaporated down to a few milliliters. The resulting concentrated solution was then evaporated to dryness in a 10-mℓ volumetric flask with purified dry nitrogen. The dry residues were made up to volume with a solution of purified n-octane-cyclohexane (9:1 v/v) and examined in the cryogenic apparatus.

The quasilinear or narrow-lined emission spectra at 77 K of each solution was recorded using an excitation wavelength of 375 nm and an emission wavelength of 403.0 nm. Studies were carried out to indicate the range of concentration over which a linear relationship existed between signal intensity at 403.0 nm and the concentration of the benzo(a)pyrene in the cyclohexane-n-octane solution. Excellent linearity was obtained between concentrations 10^{-8} to 10^{-6} *M* and good reproducibility was found for measurements in the 1 ng/mℓ to 25 ng/mℓ range.

A mean recovery of the benzo(a)pyrene of approximately 92% was obtained for six samples taken through the extraction and measurement step.

Studies by the same group were also performed to evaluate the possible quenching effect on benzo(a)pyrene from other PAH likely to be found in water samples. Interference from PAH such as indeno[1,2,3-cd]pyrene, fluoranthene, and benzo(k)fluoranthene was negligible except at benzo(a)pyrene concentrations of 10^{-6} *M* where the average decrease in signal intensity at 403.0 nm was approximately 20%. Benzo(k)fluo-

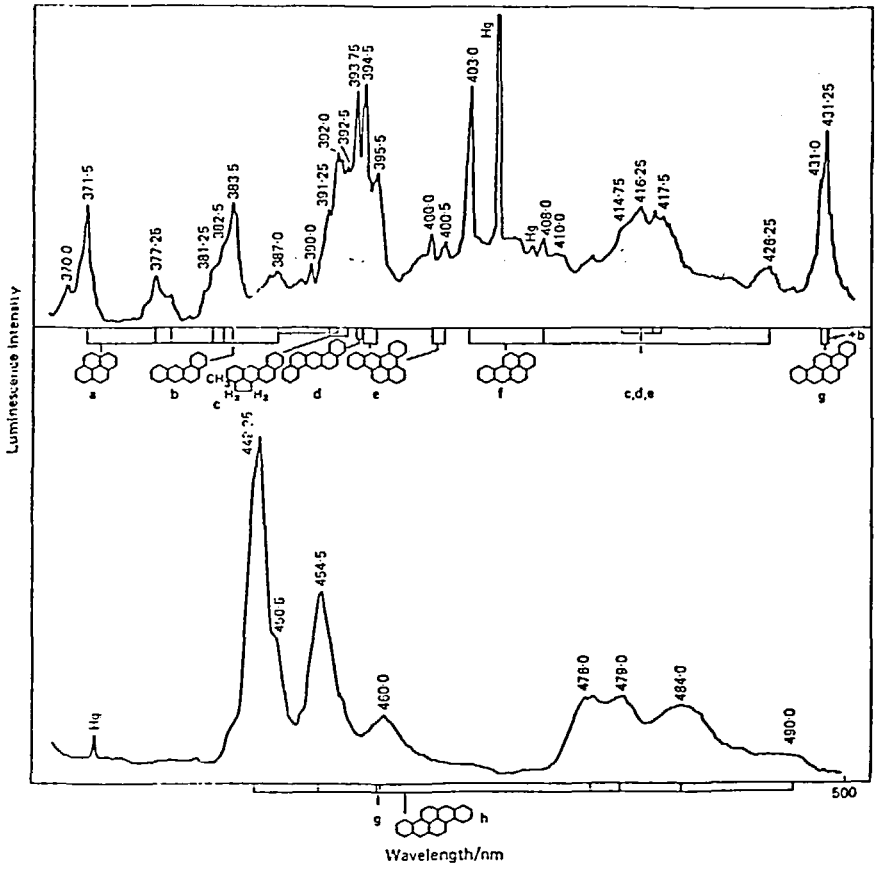

FIGURE 7. Emission spectrum of an eight-component synthetic mixture of PAH in n-octane:cyclohexane solvent at 77 K: (a) pyrene; (b) benz(a)anthracene; (c) 3-methylcholanthrene; (d) dibenz(a,h)anthracene; (e) 1,2,4,5-dibenzopyrene; (f) benzo(a)pyrene; (g) 3,4,9,10-dibenzopyrene; (h) 3,4,8,9-dibenzopyrene.

ranthene exhibits maximum emission at 403.0 nm, but at equal concentrations with benzo(a)pyrene, the former shows only approximately 10% of the latter's response at 403.0 nm.

Extracts of rain, river, and drinking water were analyzed for benzo(a)pyrene content, and the benzo(a)pyrene concentration determined by a standard addition method. The benzo(a)pyrene content of the river and rain water was found to be approximately 30 to 35 ng/ℓ and the drinking water had a greatly reduced level of benzo(a)pyrene, approximately 0.5 ng/ℓ. The total analysis time for the procedure, including the extraction, was less than 2 hr.

Colmsjö et al.[25-27] have employed Shpol'skii effect low temperature fluorescence spectroscopy to characterize PAH in HPLC eluants. The liquid chromatographic eluates from environmental samples were divided into equal fractions; most fractions consisting of 1 to 3 components at most. The fractions were collected in 3-mℓ Pyrex® tubes and the solvent was gently evaporated under a dry nitrogen stream. After transferral to the quartz cells, the residues were dissolved in n-hexane, n-heptane, or n-octane. The solutions were frozen to 63 K (solid nitrogen) and irradiated with a xenon-arc or mercury vapor source. The various components were identified by comparison of the resulting spectra with reference spectra. Identifications of isomers were much easier with this

technique as opposed to room temperature fluorescence or even mass spectrometry, because isomeric PAH gave rise to distinct quasilinear spectra. A drawback, of course, is that standard reference spectra are needed. Recent studies[27] indicate that trace components which give rise to very minor HPLC peaks can still be unambiguously identified by Shpol'skii fluorescence, even in the presence of large excesses of nonfluorescing substances.

Quantitative analyses of PAH by Shpol'skii effect fluorescence spectroscopy have not been widely reported in the literature as the result of a number of difficulties. As mentioned earlier, the range of linearity extends only over two orders of magnitude of concentration. Overlap of solute absorption spectra giving rise to "inner filter" effects and energy transfers between the solute and other species in the sample have caused inaccurate intensity values.[28] Other factors negatively influencing quantitative accuracy include formation of microcrystalline solute aggregates, different rates of freezing, fluctuations in the final solution temperature, and differences in fluorophore concentrations.[28] Although it has been possible to analyze benzo(a)pyrene successfully, its application to a mixture of many PAH is uncertain. However, Shpol'skii effect fluorescence spectroscopy should be useful if the technique can be interfaced with high performance liquid chromatography equipment. The distinctiveness of the spectra is unquestionable, and the apparatus necessary for the technique is not uncommon.

VI. MATRIX ISOLATION SPECTROSCOPY

The technique of matrix isolation (MI) developed some 20 years ago has been adapted for analytical purposes by Mamantov, Wehry, et al.[28-37] at the University of Tennessee. In the matrix isolation technique, the solid or liquid sample is vaporized and allowed to effuse from a Knudsen cell, and the gaseous sample is then thoroughly mixed with a large excess (a factor of 10^3–10^6 on a mole basis) of the matrix gas.[35] Any gas can be used as the matrix gas provided that it does not absorb in the wavelength range of interest and is chemically inert. Nitrogen has been the gas most commonly used.[35] The gaseous mixture is then condensed as a solid on a suitable optical window held at a temperature of 20 K or below. The solid sample can then be analyzed by various spectroscopic methods. The objective of the MI method is similar to the Shpol'skii technique discussed earlier, which is to minimize the interactions of the solute with the solvent so that the spectra which are observed in the solid sample resemble as closely as possible those of isolated molecules in the gas phase at low pressure. Resulting sharp-line spectra are thus useful for "fingerprinting" purposes, and Beer's law relationships are valid. PAH can be analyzed by this technique because they can be vacuum sublimed at temperatures of 25 to 200°C.[28]

The components of the apparatus that comprise the matrix isolation technique are as follows. The sample cell or Knudsen cell is a short piece of approximately 7-mm glass tubing with a vacuum joint at one end and an orifice of <0.5 mm at the other. The cell is wrapped with heating wire which is temperature controlled by a variable transformer. To achieve the required temperatures of 10 to 20 K, a small closed-cycle refrigerator is used eliminating the need for liquid refrigerants.[28,29] The optical window upon which the sample mixture is condensed must be both transparent in the appropriate wavelength range and possess high thermal conductivity. The material of choice for the UV-VIS region is sapphire and for the IR region cesium iodide is used. Further instrumental details are found in the references.[28-37]

MI fluorescence spectroscopy naturally possesses the same advantage as does Shpol'skii effect spectroscopy, e.g., a more structured and sharp-lined spectrum. Bandwidths in MI fluorescence are somewhat greater than for Shpol'skii fluorescence spectra. However, MI fluorescence studies can be carried out using n-alkane solvents

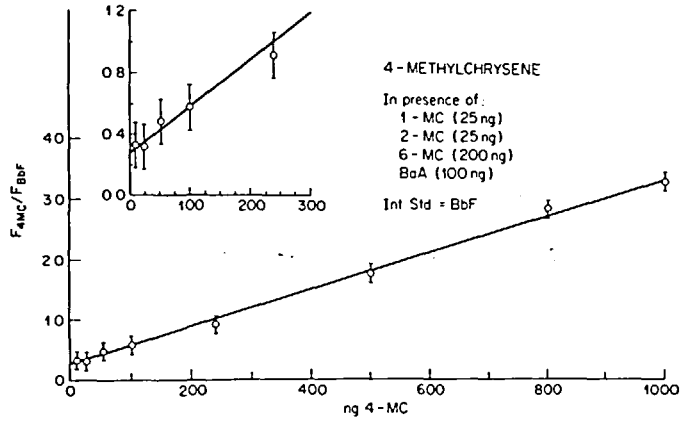

FIGURE 8. Analytical curve for matrix isolation fluorimetric determination of 4-methylchrysene in the presence of 1-methylchrysene (25 ng), 2-methylchrysene (25 ng), 6-methylchrysene (200 ng), and benz(a)anthracene (100 ng). Benzo(b)fluorene was used as the internal standard.

(introduced similarly to nitrogen as a vapor) and bandwidths are indeed smaller. MI fluorescence analyses are also aided by the use of organic solvent because there are fewer bands in the spectrum. As a result, interpretation of spectra is easier.

Quantitative analyses are performed with the use of an internal standard. The necessity of quantitative transfer of sample to cryostat window is thus eliminated provided the fraction of each sample reaching the window is identical to the internal standard. Calibration curves for individual PAH are linear from their limits of detection (approximately 5 to 50 pg) to approximately 5 μg, i.e. over five orders of magnitude in concentration.[35] In addition, the calibration curves for individual PAH in a mixture overlap those for PAH examined individually, indicating little interference from other solute species. As an example of this, Figure 8 illustrates the analytical calibration curve for 4-methylchrysene in the presence of other PAH: 1-methylchrysene (25 ng), 2-methylchrysene (25 ng), 6-methylchrysene (200 ng), benz(a)anthracene (100 ng), and benzo(b)-fluorene, the internal standard (200 ng).[31] As can be seen, the MI fluorescence calibration curve is linear over the range of 8 ng to 1 μg, even with large excesses of other PAH.

Developmental work has focused largely on the use of lasers as excitation sources. Tunable dye lasers are the most versatile. Selective excitation is extremely important since it was observed that the excitation spectra at low temperatures using organic solvents are also sharp lined.[30] Since the excitation spectrum consists of very narrow peaks, the monochromatic laser line can be centered on a sharp line specific to a particular compound in the excitation spectrum leading to a high degree of selectivity in the emission spectrum for a particular compound. A minor change of a few nanometers in excitation wavelength can result in drastic changes in spectral profile.

A number of environmental samples and chromatographic fractions have been analyzed by MI fluorescence spectroscopy. Liquid chromatography fractions from a "Synthoil" (synthetic fuel oil) sample were examined with as many as five or six PAH identified from one recorded spectrum. Analysis at several excitation wavelengths yields even more information and serves to discriminate peaks which appear to be minor or "buried" within larger peaks at other excitation wavelengths. Other samples, such as chromatographic fractions from coking plant wastewater, have been analyzed by MI fluorescence methods.

Since more than one PAH is identifiable in MI fluorescence spectra, the need for complete chromatographic resolution is lessened. However, even in cases where MI

fluorescence peaks are indistinguishable, another laser-related technique can aid in differentiation. The electronically excited states of the individual PAH molecules have finite and unique decay times, normally in the range of 10 to 100 nsec. Therefore, if two PAH are indistinguishable in a particular spectrum but have different decay time constants, the two can be more fully resolved.[7,9] An example of the usefulness of this technique can be seen in the analysis of benzo(a)pyrene in the presence of benzo(k)fluoranthene, both of which cannot be resolved easily by chromatographic means or by MI fluorescence. The time constant for benzo(a)pyrene is 78 nsec compared to 13 nsec for benzo(k)fluoranthene. If the fluorescence measurement is performed with a delay time of 90 nsec, measurement of the 13 ng of benzo(a)pyrene can be measured in the presence of 1 μg of benzo(k)fluoranthene, since the latter's intensity decreases considerably compared to benzo(a)pyrene. Further details on the method are found in Reference 34.

The properties of lasers have proven useful in another area of MI fluorescence. The bandwidths of the fluorescence spectral lines can be reduced by laser excitation.[36] The procedure is known as either "site-selection spectroscopy" or "fluorescence line-narrowing spectroscopy". The laser excitation source must have an output with a bandwidth smaller than the bandwidth of the fluorescence peak width. The effect is such that only a fraction of the PAH molecules are excited (those occupying nearly identical sites), and the resulting "site selected" fluorescence spectrum is much narrower than if a normal broadband excitation source was used.[9] The objective of further research will be to improve the spectral resolution and achieve the same resolution with nitrogen that is obtainable with organic solvents.

Infrared spectroscopic methods have generally been ignored in PAH analyses as a result of poor sensitivity and the difficulties inherent in quantitative analyses by IR methods. However, the development of Fourier transform spectroscopy has reduced detection limits to the nanogram level.[32] A further difficulty with IR methods has been the problem of preparing reproducible samples (e.g., with KBr discs, mulls, etc.). These problems are avoided using MI techniques. With the proper choice of the matrix gas, the sample matrix can be transparent throughout the entire wavelength region, and due to the homogeneous dispersion of solute molecules in the matrix gas, Beer's law should be applicable.

MI-FT-IR spectra of chrysene, benz(a)anthracene, triphenylene, pyrene, and benzo(a)pyrene are shown in Figure 9.[28] Nitrogen was the matrix gas (matrix/sample ratio approximately 1000:1), and the 1500 to 500 cm^{-1} wavenumber region was scanned. A close-up of the 900 to 700 cm^{-1} region of the spectra is shown in Figure 10 where it is obvious from the spectra that enough differences exist to allow accurate qualitative analysis. The absorption bands are relatively narrow (4 to 8 cm^{-1} maximum bandwidth). The IR bandwidths can be decreased at the cost of decreased sensitivity by increasing the matrix/sample ratio of 50,000 or 100,000:1.[28] Single peaks are often resolved into two or more component peaks.

Mixtures of up to ten PAH can be qualitatively identified by MI-FT-IR. A spectrum of a five-component PAH mixture is shown in Figure 11. Each component can be identified by at least one unique absorption band in the 900 to 700 cm^{-1} region. For more complex samples, initial preseparations are necessary. Chromatographic methods are one approach to accomplish this. Another method of achieving a prefractionation could be based on the differences in sublimation temperatures of the various PAH .[36] A low temperature (25 to 75°C) spectrum contains most of the lower molecular weight components, while the (100 to 200°C) fraction contains the high molecular weight PAH. More information on the use of fractional sublimation as an aid to MI analyses is found in Reference 36.

One of the most valuable applications of MI-FT-IR is the analysis of mixtures of

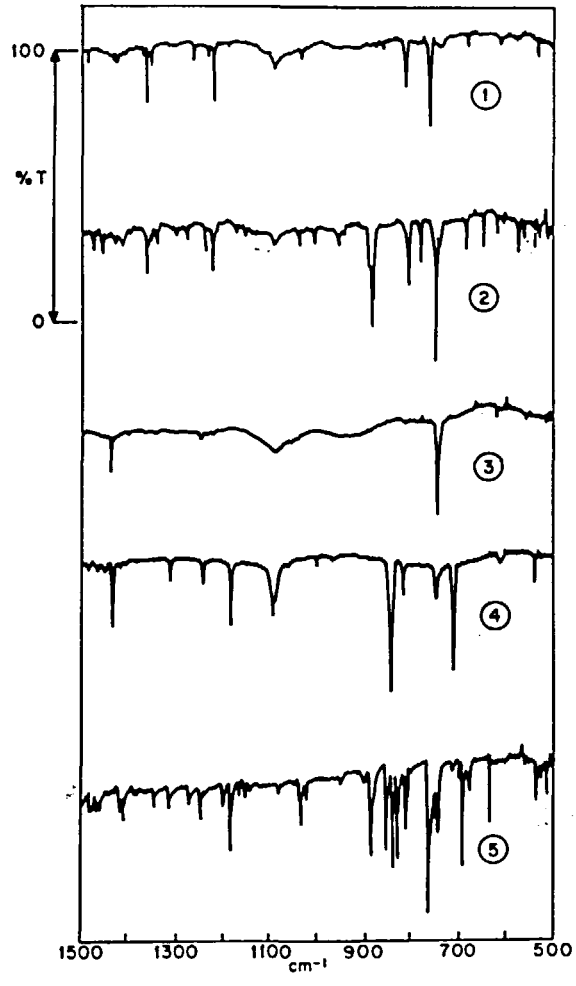

FIGURE 9. MI-FT-IR spectra of (1) chrysene; (2) benz(a)anthracene; (3) triphenylene; (4) pyrene; and (5) benzo(a)pyrene in a nitrogen matrix (nitrogen/PAH mole ratio approximately 10^3). Resolution: 2.0 cm^{-1}. Spectra are plotted vs. the vacuum reference. Note that the 1100 cm^{-1} peak is caused by an impurity on the cesium iodide window.

isomeric PAH, because chromatographic methods cannot at this stage provide complete resolution of many complex environmental samples. For instances, the compound 5-methylchrysene is a powerful carcinogen, equal in potency to benzo(a)pyrene, while the other five methylchrysenes are less carcinogenic.[31] Therefore, quantitation of each isomer is important in order to avoid an overestimation of the carcinogenic potential. MI-FT-IR spectra of these six isomers are illustrated in Figure 12, while the major absorption bands are listed in Table 6. Each spectrum was obtained by 1000 individual scans at a resolution of 1 cm^{-1}. The matrix gas was nitrogen with a matrix-to-sample ratio of 10^5:1. The spectra cover only the 700 to 900 cm^{-1} region since according to the authors,[31] all the intense IR bands useful for fingerprinting are found in that region.

The MI-FT-IR spectrum of a mixture of the 1-, 2-, 3-, and 5-methylchrysenes is shown in Figure 13. The 4- and 6-methyl isomers do not have any unique bands which do not overlap bands from the other four isomers. However, the 4- and 6-methyl-

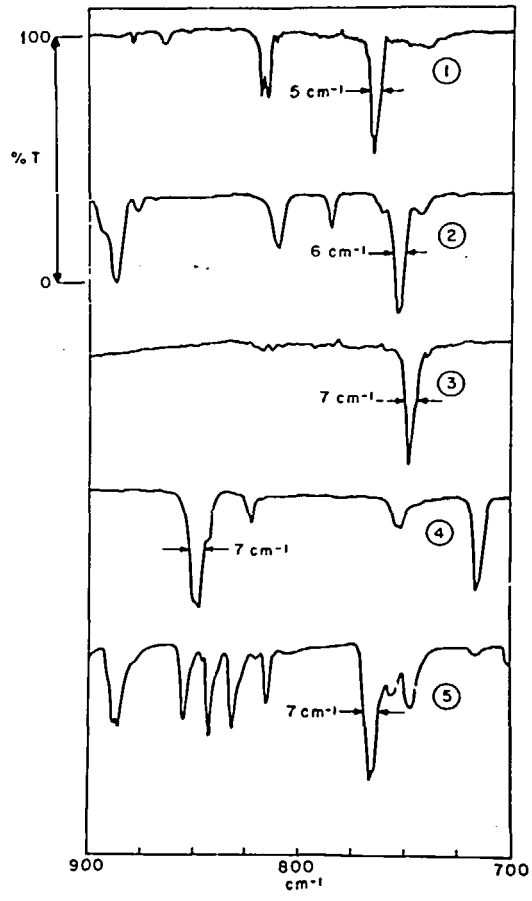

FIGURE 10. Expanded spectra in the region 900 to 700 cm^{-1} for the same compounds as in Figure 9.

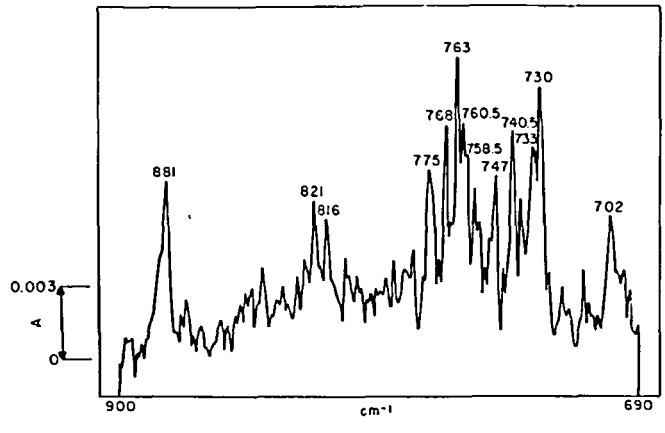

FIGURE 11. The MI-FT-IR spectrum of a five-component synthetic mixture of PAH consisting of 7 μg of benzo(a)fluorene (753, 760.5 cm^{-1}), 7 μg of benzo(b)fluorene, (775 cm^{-1}), 7 μg of triphenylbenzene, (758.5, 702 cm^{-1}), 10 μg of anthracene (733, 730 cm^{-1}), and 10 μg of phenanthrene (740.5 cm^{-1}) in nitrogen (range of nitrogen/PAH mole ratio between 1.46×10^5 to 3.56×10^5). Resolution: 2.0 cm^{-1}.

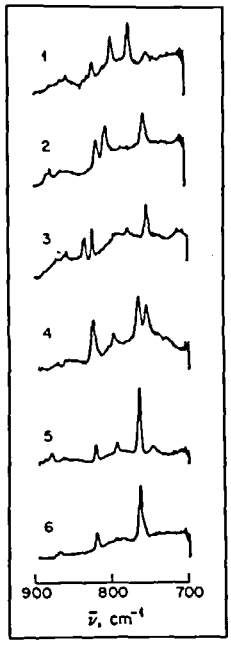

FIGURE 12. MI-FT-IR spectra of the six methylchrysenes in the 700 to 900 cm^{-1} region at 15 K. Sample:matric ratio = 1:1 × 10^5.

Table 6
MI-IR ABSORPTION BANDS OF METHYLCHRYSENES

(700 to 900 cm^{-1} Region; Resolution:1 cm^{-1})

Compound	Absorption peaks (cm^{-1})
1-Methylchrysene	822, (797, 795.5 doublet), 774[a]
2-Methylchrysene	880, 818, 805, 754.5[a]
3-Methylchrysene	(865.5, 862 doublet), (839, 836.5 doublet), 827.5, 774, 754.5[a]
4-Methylchrysene	828[a], (800, 798 doublet), 767.5, 756[a]
5-Methylchrysene	(892, 883 doublet), (864, 867 doublet), 824, 795.5, 765[a], 748
6-Methylchrysene	823.5, 764.5[a]

[a] Indicates strongest peak.

chrysenes can be identified in a six-component mixture by MI fluorescence spectroscopy, which points to the necessity of using these techniques in a complementary fashion.

The Beer's law plots for the FT-IR absorbances are linear from the limits of detection (200 ng) to 5.0 μg, with a relative standard deviation of 8.4% for the individual absorbances when taken as a ratio to the internal standard of triphenylene.

In a similar series of studies Hinton et al.[33] were able to analyze mixtures of naphthalene, 1- and 2-methylnaphthalene along with five of the dimethylnaphthalenes by a MI-FT-IR method.

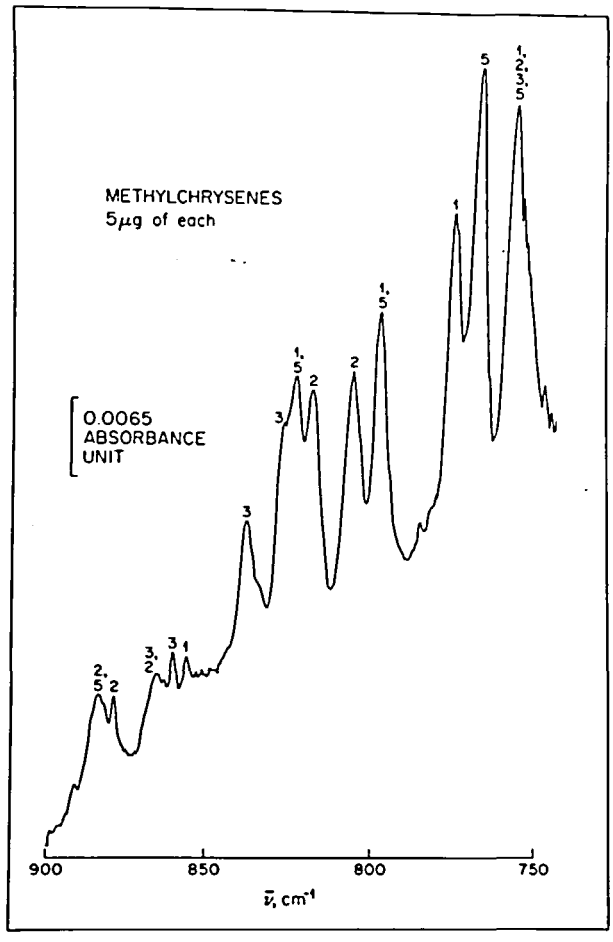

FIGURE 13. MI-FT-IR spectrum of four-component methylchrysene mixture (1-, 2-, 3-, and 5-isomers; 5 μg of each) at 15 K. Sample:matrix ratio = 1:3.3 × 10^4.

One of the main objectives of the MI methods has been to develop a procedure to analyze simple mixtures of PAH and to resolve isomeric PAH with high sensitivity and efficiency. The logical primary goal would be to apply the technique to the analysis of chromatographic fractions, and quite significantly, reduce the necessity of achieving complete chromatographic resolution, since MI is capable of resolving and identifying isomeric PAH (including those difficult to resolve by mass spectra).

Sampling in MI requires a gaseous state, suggesting that MI spectroscopy might be advantageously coupled to a gas chromatograph. There are a number of requirements which must be met by MI spectroscopy before qualifying as an efficient gas chromatographic detector.[36] The first is that the spectroscopic measurement be rapid, approximately 30 sec or less for packed column GC and of the order of a few seconds with capillary GC. Therefore, an entire spectrum must be obtained within this time interval. Fourier transform methods are naturally well suited for such rapid measurements. For fluorescence measurements, however, the task is not as simple. Conventional spectrofluorimeters obtain a spectrum by scanning with a diffraction grating. If high spectral resolution is essential scan times of 5 to 20 min are necessary, which makes them useless for GC detection. There are two types of electronic "array detectors" (TV cameras and

solid-state diode arrays) which obviate this problem.[36] These detectors are electronic analogues to a photographic plate detector in classical spectrographs. They do not require mechanical scanning in order to produce spectra.

In addition, there are other problems. The GC eluant is generally at a high temperature, while MI is a cryogenic method. Furthermore, the chromatographic separation of complex environmental samples may require hours. These problems are being addressed by proper cryostat and interface designs and by the use of movable surfaces upon which the sample can be condensed.

The problems with interfacing high pressure liquid chromatography to MI methods are naturally more severe due to the liquid eluant and as such are in need of much more development. No literature references are yet available on this.

MI spectroscopy is a proven technique for both qualitative and quantitative analyses of simple mixtures of PAH. MI could also be used with other spectroscopic methods such as phosphorimetry, Raman methods, and EPR techniques. With the advent of commercially available closed-cycle refrigerators capable of providing temperatures of 15 to 20 K, the two complementary low temperature fluorescence methods have approximately equal instrumental requirements. We would, therefore, suggest MI methods over Shpol'skii, especially since MI methods can use organic solvents and in essence mimic the selectivity and high resolution characteristic of the Shpol'skii technique.

VII. DERIVATIVE AND WAVELENGTH MODULATION SPECTROMETRY

A. Derivative Methods

The derivative spectroscopic technique depends upon the measurement of the first or higher derivative of the luminescence intensity or absorbance with respect to wavelength, $dI/d\lambda$, $dA/d\lambda$, $d^2I/d\lambda^2$, etc. This quantity is then plotted or scanned vs. wavelength. The advantage of the method is that it is possible to resolve or enhance small peaks that are incompletely resolved from larger peaks. A number of researchers have successfully applied derivative spectroscopy to the analyses of PAH.[38-43]

O'Haver[38] has summarized the experimental methods for obtaining derivative spectra. In the case of a spectrum obtained point by point or digitally, the differentiation of the signal can be carried out numerically. Otherwise, for real-time application the experimentally available techniques for generating derivative spectra fall into two basic classes:[38] those systems which operate on the output signal of the spectrometer (electronic differentiation) and those systems which operate on the light beam in the optical network of the instrument (wavelength modulation).

The electronic mode is based on the assumption that if the wavelength scan rate, $d\lambda/dt$, is constant, then the derivative of intensity with respect to wavelength, $dI/d\lambda$, is proportional to the derivative of intensity with time, dI/dt. This can be expressed in equation form as

$$\frac{dI}{d\lambda}\frac{d\lambda}{dt} = \frac{dI}{dt} \tag{2}$$

and since $d\lambda/dt$ = const.

$$\frac{dI}{d\lambda} = \frac{1}{\text{const.}}\frac{dI}{dt} \tag{3}$$

The derivative, dI/dt, is easily measured with operational amplifier circuits with appro-

priate resistor-capacitor RC feedback circuits[38] and the constant is determined from knowledge of the wavelength scan rate.

By modulation of the wavelength of measurement over a fixed interval, $\Delta\lambda$ (small compared to the spectral band width), the amplitude of the resulting modulated intensity will be directly related to the slope of the spectrum within $\Delta\lambda$ and thus be a measure of the first derivative. If the detection system is frequency selective, e.g., a lock-in amplifier, the modulated intensity corresponding to the first and second derivative can be measured. To measure the first-order derivative, the amplifier is set to the frequency of the wavelength modulation, while the second-order derivative can be obtained by tuning the amplifier to twice the modulation frequency. Experimental details on the technique can be found in References 38 and 39.

In absorption measurements wavelength modulation generally leads to the higher signal-to-noise ratio, whereas the two methods are comparable in luminescence measurements.[38] The electronic differentiator is simpler, less expensive, and can record zero, first-, and second-order derivative spectra simultaneously. On the other hand, it cannot obtain derivatives at a fixed wavelength, and the scan rate must be constant throughout the scan and reproducible from scan to scan. These problems are not faced with the wavelength modulation method.

Gammage et al.[6] have published a number of industrial and environmental monitoring applications carried out with a second derivative spectrometer. One application is based on the measurement of naphthalene and alkylnaphthalene vapors which arise from many fossil fuel processing operations. A complete resolution of peaks resulting from a mixture containing 90 ppb of naphthalene and 73 ppb of 2-methylnaphthalene can be seen in Figure 14. This separation can be observed in the second derivative spectrum despite the fact that the two peaks are only 3 nm apart.

Vo-Dinh et al.[15,43] have used the second derivative technique to increase the selectivity of synchronous spectroscopy and room temperature phosphorimetry analyses. The analysis of a mixture of phenol and the isomeric cresols was facilitated by use of the second derivative technique. Another advantage of the technique was found for analyses of turbid samples likely to be encountered in environmental samples. A turbid sample generally gives rise to an absorption which slowly varies with wavelength so that the second derivative spectrum trace will be relatively free from contributions due to sample turbidity.

O'Haver[38] described a number of applications of derivative spectroscopy to PAH mixtures. In a sample containing 1.0 ppm anthracene, 21 ppb chrysene, and 9 ppb carbazole, the normal fluorescence emission band exhibited only a weak shoulder as a result of the chrysene. However, with the use of second derivative spectroscopy, as little as 6 ppb of chrysene can be quantitatively measured. A number of other binary and ternary PAH mixtures were examined using derivative methods.

B. Selective Modulation Techniques

Figure 15 is an illustration of the basic principle upon which selective modulation methods developed by O'Haver et al.[38,39,44] are based. The overlapping excitation spectra of two hypothetical substances A and B are shown in Figure 15. A simultaneous fluorescence analysis of A or B is impossible because of the overlap of the spectra. Selective excitation which involves excitation at a wavelength unique to only one solute is not practical. In the selective modulation technique, the wavelength of the excitation monochromator is allowed to periodically modulate over the indicated wavelength interval, $\Delta\lambda$. The modulated excitation wavelength gives rise to modulated emission intensities and with $\Delta\lambda$ positioned as shown, the emission of component B is modulated at the wavelength modulation frequency, 1F, while the emission of component A will naturally be modulated at the frequency 2F. If the detection system includes a frequency

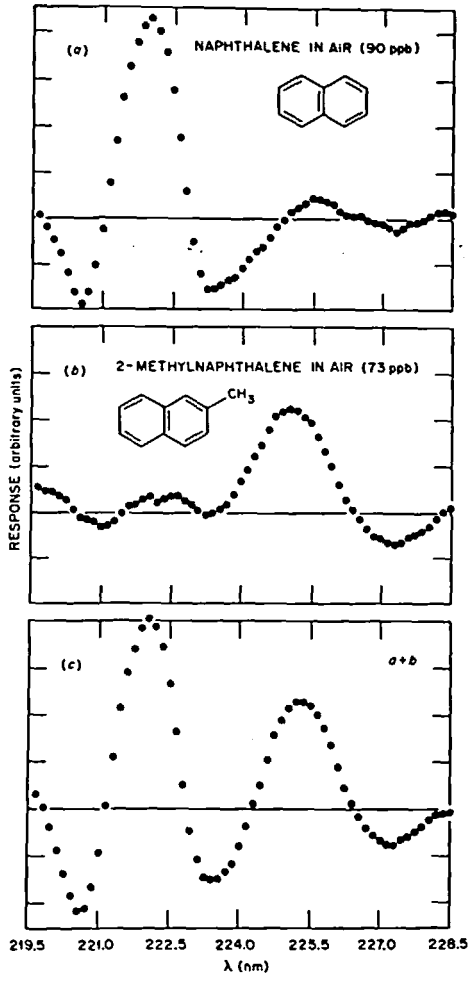

FIGURE 14. Second derivative spectra of naphthalene and 2-methylnaphthalene in air.

selective lock-in amplifier, the emission of either A or B can be selectively monitored independently. Therefore, the emission of either A or B can be measured without interference from the other. The complete principles and further details of the technique can be found in Reference 44.

Selection and positioning of the $\Delta\lambda$ parameter depends on the particular PAH involved. Details can be found in the References,[38,44] but the critical requirement which must be met is that the two excitation spectra not have exactly coincidental maxima and minima at all points. Modulation conditions can be predicted in advance but are usually determined by running a standard sample to optimize the parameters.

In general, there are two different modes of operation. There is a "null mode" in which the spectrum of one component in a binary mixture is nulled or erased.[44] The other is an enhancement mode which can be used to selectively increase the intensity of one substance relative to others and is applicable to multicomponent mixtures. However, mixtures of more than three components become quite difficult to handle.

Figure 16 is a schematic of the apparatus required. A normal fluorescence spectrometer can be modified to allow modulation techniques to be carried out. Conventional

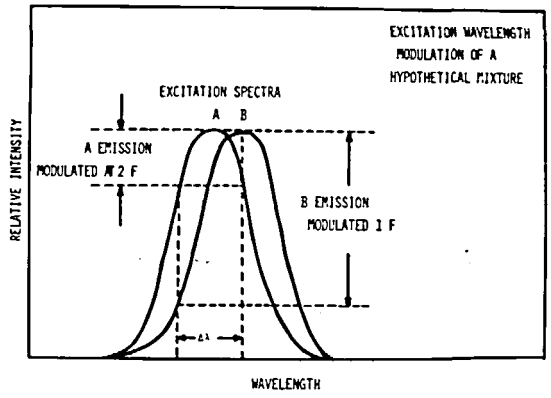

FIGURE 15. The principle of selective modulation. A and B are the excitation spectra of two hypothetical molecules in a mixture. Modulation of the excitation wavelength over the indicated interval $\Delta\lambda$, at a modulation frequency of F hertz, will modulate the fluorescence intensity of B at the same frequency, while the fluorescence intensity of A will be modulated at twice this frequency (i.e., 2F).

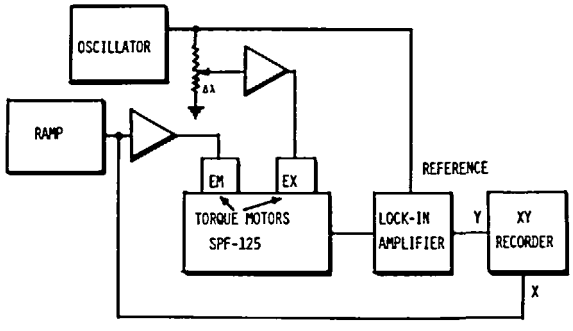

FIGURE 16. Block diagram of the selective modulation system in the excitation modulation mode; for operation in the emission modulation mode, the control lines to the torque motors are interchanged.

scanning and modulation techniques are accomplished by application of the appropriate ramp and AC waveform to the motors.[44] Complete instrumental details can be found in References 38 and 44.

The techniques have been applied to mixtures of PAH. O'Haver et al.[38,39,44] have successfully determined benz(a)anthracene concentrations in the presence of an excess of chrysene. Various other model PAH mixtures can be analyzed in like fashion, e.g., pyrene-anthracene, chrysene-benz(a)anthracene-triphenylene, and chrysene-anthracene. The emission spectrum of pure chrysene excited at an excitation maximum is shown in Figure 17A. Upon addition of a large excess of anthracene, the resultant emission spectrum, Figure 17B, is obtained by selective excitation at a wavelength yielding the greatest chrysene to anthracene intensity ratio. It can be seen that the effect of the anthracene is significant enough to distort the spectrum from the spectrum of pure chrysene, in Figure 17A. However, the null-mode selective modulation designed to strip out the anthracene spectrum is successful in restoring the original emission

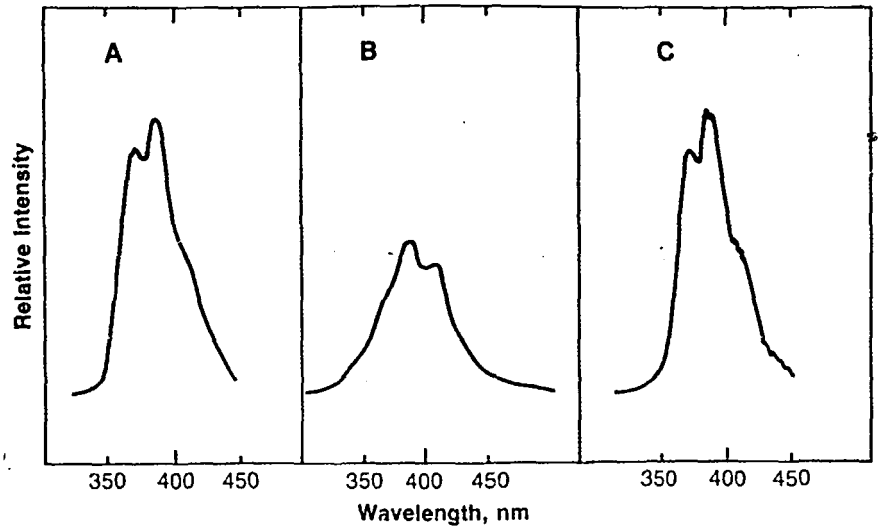

FIGURE 17. Excitation wavelength modulation to allow measurement of fluorescence emission spectrum of one component in a binary mixture of PAH whose excitation and emission spectra are extensively overlapped. (A) Emission spectrum of a pure solution of chrysene excited at its excitation maximum. (B) Emission spectrum of a mixture of chrysene in the presence of an excess of anthracene excited near an excitation minimum of anthracene. (C) Emission spectrum of the same mixture obtained by excitation wavelength modulation with conditions adjusted to strip out the spectrum of anthracene.

spectrum of chrysene, Figure 17C. The technique cannot result in any signal-to-noise ratio gain, and the intensity of Figure 17C is obtained only by increasing instrumental sensitivity.

One of the apparent difficulties is this reduction in the signal-to-noise ratio. In Figure 17 the contribution of the noise is obvious. In addition, selective modulation does not alter in any way fluorescence quenching, inner filter effects, and other variables which affect the slope of the analytical curve.[39] However, as a technique which requires only a moderate amount of instrumental modification, provides real-time results without computer assistance and is proven in its ability to add increased selectivity in PAH analyses, selective modulation will find use in future applications.

VIII. DEVELOPING TECHNIQUES

A number of diverse spectroscopic analytical methods have been presented for use in improving existing chromatographic detectors and as convenient techniques for providing relatively rapid analysis of simple mixtures of PAH. In addition to the established analytical procedures which have already been discussed, a large number of developments based on spectroscopic principles are in progress and these may find future application.

A. Sensitized Fluorescence Detection of PAH

None of the chromatographic and spectroscopic techniques for the analysis of PAH provide the analyst with a low cost, rapid, and highly sensitive means for screening environmental samples for PAH. Such a technique would have to involve only readily available instrumentation and give a rapid yes or no answer as to the presence of PAH at the lowest detectable level of the more sophisticated analytical methods, e.g., 1 to

10 pg. This capability would reduce the time and money required to apply more sophisticated analytical procedures on a routine basis to samples which may not contain any detectable level of PAH. Research at the Arthur D. Little laboratories into the phenomenon of sensitized fluorescence may provide the needed capability.[45,46]

The process of directly excited fluorescence involves the absorption of radiation by the PAH molecule thus raising the species to any number of excited singlet states. Upon an energy release to the lowest vibrational level of the excited singlet state, the fluorescence emissions occur as the molecule returns to any number of ground-state vibrational levels. If a second compound is present, a vibrational coupling interaction may occur between the excited states of the two molecules which results in a resonant energy transfer and subsequent vibrational deactivation and fluorescence emission from the second compound. This second compound must have a common vibrational frequency with the first compound and possess the lower energy vibrational ground level of the two excited singlet states. The transfer of resonant energy is most efficient when this second compound is present at an extremely low molar ratio to the original compound or donor compound. Examples of sensitized fluorescence include the detection of naphthacene in benz(a)anthracene and in anthracene at molar ratios of 10^{-4} and 10^{-6}, respectively.[46]

Limits of detection for PAH for direct fluorescence on TLC plates are approximately on the order of 1 to 10 ng. With sensitized fluorescence detection using the analyte as the "minor" component one would expect an eventual reduction in limits of detection by a factor of 10^4-10^6 as noted in the earlier example of naphthacene systems.

The absorption and fluorescence of PAH in general shift to longer wavelengths (lower energy) with increasing conjugation and molecular weight. Absorption and fluorescence also occur at longer wavelengths for linear as opposed to nonlinear PAH, e.g., anthracene fluoresces in the visible while phenanthrene exhibits only ultraviolet fluorescence. On this basis a general rule was devised by Smith and Levins[45,46] that lower molecular weight PAH could sensitize higher molecular weight PAH analytes, and for isomers, nonlinear compounds should sensitize a linear compound.

Smith and Levins[46] considered only analytes which fluoresced in the visible region. Some of the model analytes and their emission peaks (nm) are anthracene-378, 400; pyrene-370, 382; benzo(a)pyrene-392, 416; dibenzo(a,i)pyrene-420, 450. Of these, benzo(a)pyrene and dibenzo(a, i)pyrene are the only two known carcinogens. In principle any PAH of interest could be examined provided an efficient sensitizer existed and detector was available. In addition, heterocyclic compounds are amenable to the technique allowing the benzocarbazoles and other compounds to be detected.

The lower molecular weight PAH tested as sensitizers included benzene, naphthalene, fluorene, and phenanthrene. Benzene was an efficient sensitizer but inconvenient due to its volatility and the subsequent need to work at lower temperatures. Theoretically,[46] the resonant energy transfer is maximized when sensitizer and analyte possess similar crystalline structures. However, phenanthrene and fluorene were rejected for use after repeated attempts to remove interfering fluorescent impurities failed. The final determinations were carried out using naphthalene which has a range of emission from 300 to 365 nm.

The substrate for the analysis is filter paper, in particular, Whatman No. 42. The other necessary supplies are naphthalene solution (60 $\mu g/\mu \ell$) in methylene chloride, pipets (Drummond Microcaps, 1 $\mu \ell$), and an ultraviolet source — 254 nm. With a pencil, three circles approximately 1 cm in diameter are marked in a row on the filter paper. One-$\mu \ell$ portions of the PAH samples are applied to two of the spots and allowed to dry. In like fashion, 1 $\mu \ell$ of the naphthalene solution is applied to the blank circle and one of the sample spots. When the spots are dried, they are viewed with the 254-nm ultraviolet source. Sensitized fluorescence will be indicated by differences in intensity and color between the sample + sensitizer spot and the two individual sensitizer and sample spots.

Approximate PAH levels which can be detected by this procedure are estimated as follows:[46]

No sensitized fluorescence — <1 pg
Weak sensitized fluorescence — 1 to 10 pg
Strong sensitized fluorescence
(but not self-fluorescence) — <100 pg
Self-fluorescence — >10,000 pg

Most of the specific detection limits for individual PAH were approximately 10 pg/spot with the limit for benzo(a)pyrene an order of magnitude lower, 1 pg/spot.

A number of other organic solutes, e.g., n-alkanes, chloroalkanes, alcohols, ethers, and nitrobenzenes were found to give no sensitized fluorescence.

The procedure was evaluated with a number of environmental sample extracts.[46] Cross-check analyses on these same samples were performed by direct-probe low-resolution mass spectrometry. In every case where the mass spectrometric technique indicated the presence of PAH, the spot test was also positive, and in two cases the spot test indicated PAH not detected by mass spectrometry. Concentrations ranged from 0.1 to 10 ng for all PAH. The very high molecular weight PAH (molecular weight > 300) are those most likely to be missed in gas chromatographic/mass spectrometric (GC/MS) analyses. Semiquantitative estimates of PAH levels in environmental extracts from wood stove emissions and coal-tar shampoo have also been accurate when verified later in the laboratory by GC/MS analysis.

Preliminary testing clearly indicates the valuable potential of sensitized fluorescence for the screening of environmental samples for PAH. The spot test can be taken out into the field and used directly on some samples without the need for extensive clean-up or preconcentration. A technique that is so simple and requires apparatus every laboratory possesses is a valuable addition. In this case, the usefulness is obvious for even greater reasons. First, the limits of detection are better than any sophisticated laboratory technique, which means the risk of screening a sample containing PAH and reporting a negative result is unlikely and second, the technique is portable which eliminates the problems of extraction, preconcentration, and sample storage and handling. The technique of sensitized fluorescence should be useful in future environmental analyses for PAH in water.

B. X-Ray Excited Optical Luminescence Spectroscopy

D'Silva et al.[47-49] at Iowa State have reported the results of analyses for PAH in environmental samples by X-ray excited optical luminescence (XEOL) spectroscopy. The actual analytical procedure resembles a Shpol'skii effect analysis; however, X-radiation is used for the excitation rather than UV.

After preconcentration and initial fractionation, the sample extracts were dissolved in n-heptane and cooled to 90 K with liquid nitrogen prior to irradiation. The primary X-radiation was obtained from a tungsten target X-ray tube under conditions to give X-rays in the range of 1 to 10 Å. The experimental procedure is reported in more detail in Reference 47.

An independent evaluation of the relative advantages of X-radiation and UV radiation for excitation is not yet available. Some of the reasons stated by D'Silva et al.[47] for using X-rays include the freedom from optical cross-talk between the exciting and luminescence radiation and the ability of X-rays to populate electronic levels in molecules not accessible by UV radiation. Therefore, less unwanted interference and more spectral components would be expected. Another advantage cited[47] is the observation of substantial phosphorescence even when the phosphorescence is not observed with UV excitation.

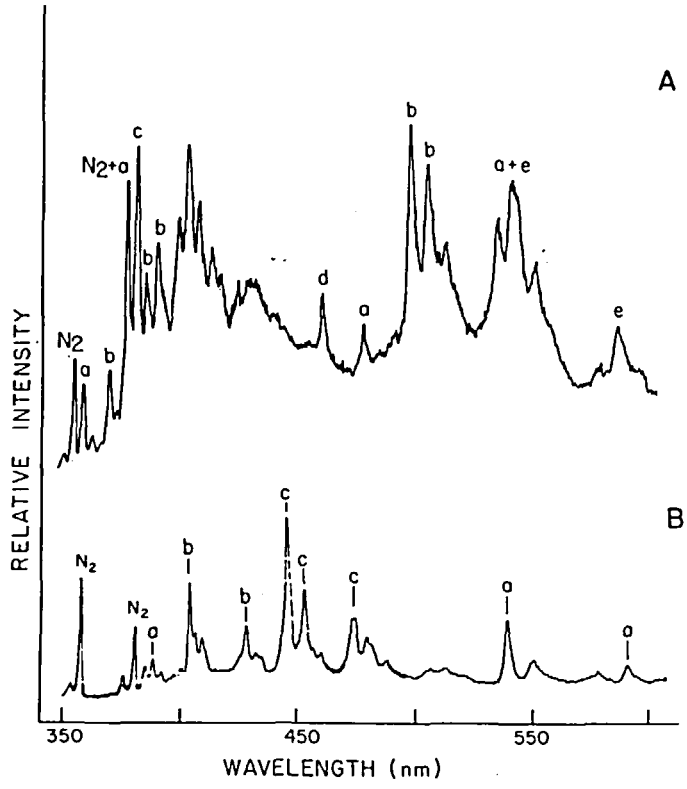

FIGURE 18. X-ray excited optical luminescence of isomeric PAH in n-heptane. The characteristic spectra of the following compounds are observed. In A: (a) chrysene, (b) pyrene, (c) benz(a)anthracene, (d) triphenylene, (e) fluoranthene. In B: (a) benzo(e)pyrene, (b) benzo(a)pyrene, (c) perylene.

An examination of the reported applications to PAH analyses suggests that the technique will be best suited for differentiation of isomeric PAH, possibly in liquid chromatographic fractions. Excellent success was evident in the qualitative and semiquantitative determination of isomeric PAH of molecular weights 228 and 252. Figure 18 is an XEOL spectrum of a number of isomeric PAH in n-heptane. All of the components can be identified. Extracts from shale oil, air particulates, and coal-tar products have also been analyzed by XEOL spectroscopy. Figure 19 illustrates the characterization of PAH in various fuel oils.[49] As with the Shpol'skii effect it has not been possible to obtain good quantitative analyses. The method of standard additions is essential and the range of linearity for calibration curves is limited. The limits of detection are on the nanogram level.

No real advantages can be seen by XEOL spectra over UV excited low temperature spectra, but the advantages noted earlier should be explored further. Instrumentally, there is some greater complexity in using X-radiation, however, the choice of UV vs. X-radiation for excitation is up to the individual analyst.

C. Fluorescence Line-Narrowing Spectrometry (FLNS)

Brown et al.[50,51] at Iowa State have introduced another form of low temperature fluorescence spectrometry. This variation involves the irradiation of PAH molecules trapped in glassy matrices formed by cooling various aqueous-organic mixtures to liquid helium temperatures (~4 K). Tunable laser sources are used as light sources to

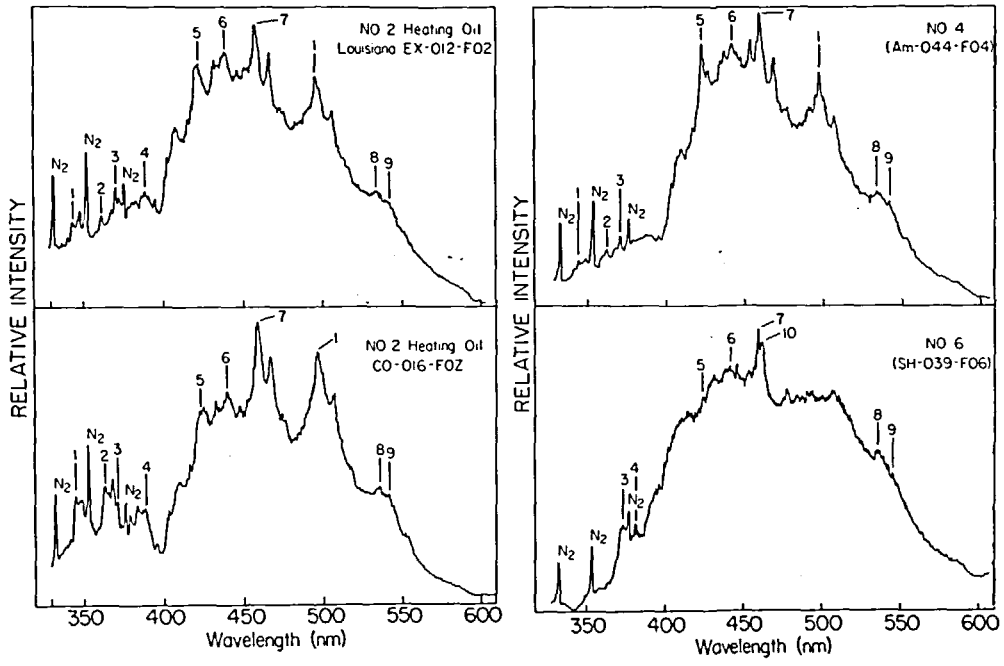

FIGURE 19. X-ray excited optical luminescence spectra of fuel oils (each sample represents 0.2 mℓ of 5 mg/mℓ fuel oil in n-heptane). (1) Phenanthrene, (2) chrysene, (3) pyrene, (4) dibenzanthracene, (5) anthracene, (6) perylene, (7) triphenylene, (8) benzo(e)pyrene, (9) fluoranthene.

insure the necessary selectivity. The technique gives rise to very narrow emission lines because the laser-induced excitation will only occur for a small subset of solvent matrix configurations where the solute species occupy sites such that their absorption profiles overlap the laser profile. The result is spectra with line widths narrow enough to allow independent characterization of several components in a mixture.

A few environmental samples have been analyzed for individual PAH, e.g., pyrene and anthracene, by FLNS. When the concentrations of the PAH in the water samples are sufficiently high to fall within the detection limits of the method (~ 10 to 100 ppt) after dilution in the glassy matrix, preseparation procedures can be avoided since the matrix can be composed of aqueous-organic mixtures.[50,51] This advantage can be quite important.

Several important advantages exist over the use of Shpol'skii matrices.[50,51] One is the range of linearity for the method. Peak heights vs. concentration of pyrene and anthracene were linear from approximately 1 ppb to over 3000 ppb, i.e., over three orders of magnitude. The second advantage involves the solvent matrix. The Shpol'skii effect spectra depend heavily on the n-alkane solvents employed, whereas in FLNS the solvent matrix composition is relatively unimportant. Water can be tolerated and a wide variety of organic solvents can also be used. Another advantage involves the time required for analysis. Fifteen minutes is the time required for formation of a reproducible glassy matrix, thereby allowing a greater number of samples to be characterized. FLNS appears to be capable of every attribute possessed by the other low temperature luminescence techniques and should therefore be further explored.

D. Developments in Laser-Induced Molecular Fluorescence in Solution at Room Temperature

Laser-induced molecular fluorescence of solutions at room temperature improves

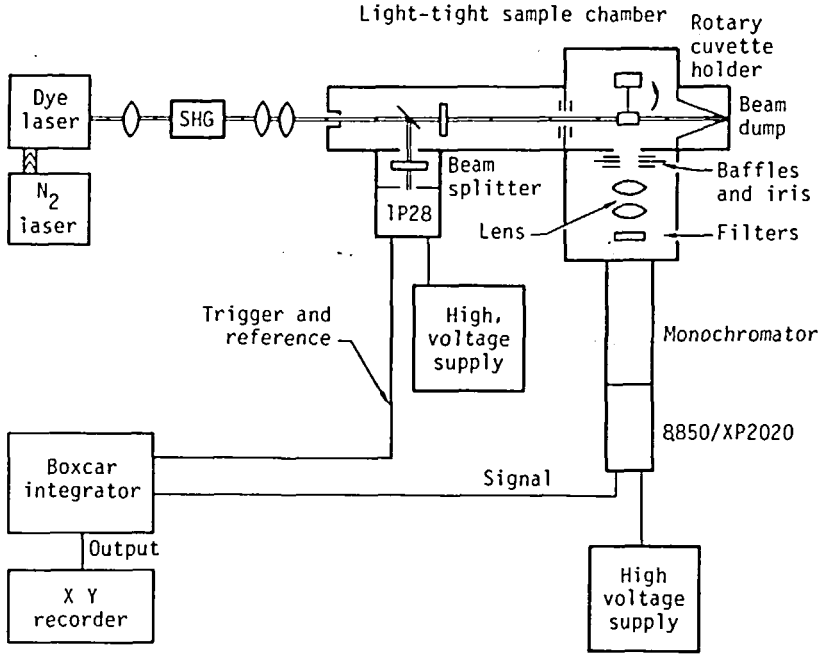

FIGURE 20. Schematic of the laser-excited spectrofluorimeter designed by Richardson et al. (see Reference 52 for further details).

the lower limit of detection for the PAH over that obtainable with nonlaser light sources. It does not, however, improve on the selectivity. Because many of the newer chromatographic columns resolve most of the isomeric PAH, the increase in sensitivity without concomitant increase in selectivity may in some cases be sufficient for an improved analysis. An added benefit to laser-induced molecular fluorescence is that cryogenic temperatures are not needed thus making it a convenient detector for high pressure liquid chromatography.

Richardson et al.[52-54] have studied laser-induced molecular fluorescence of PAH in solution at room temperature. A number of experimental configurations was tested and one of the most successful is shown in Figure 20. The excitation source was a Molectron UV 1000 N_2 laser and a Molectron dye laser operated in the DL 200 configuration. The nitrogen laser is used to pump the dye laser. Experimental details on the other laser configurations and the complete optical apparatus can be found in References 52 and 53.

The PAH were studied in methanol and methanol/water solutions. Plots of fluorescence intensity vs. concentration were found to be linear for as much as seven orders of magnitude in concentration (Figure 21). The linearity for naphthalene in Figure 21 extends from 10^{-5} *M* to less than 10^{-11} *M*. The limits of detection were on the order of picograms for the PAH studied; pyrene, anthracene, fluoranthene, and naphthalene.[52]

The low limits of detection permit the laser-induced fluorescence technique to be conveniently coupled with a high pressure liquid chromatograph.

Detection devices can help to add the needed selectivity when the chromatographic resolution is lacking. Richardson et al.[54] have used the technique of time-resolved fluorescence detection in conjunction with a laser-excited fluorescence detector. In this manner, only PAH with sufficiently long lifetimes will be measured. Other detector devices such as the derivative and wavelength modulation techniques can also add selectivity to the room temperature fluorescence detector. Detectors which can avoid

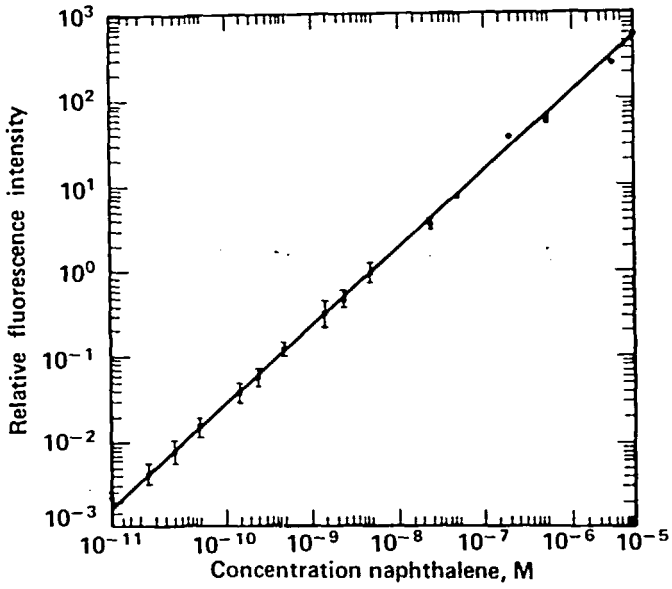

FIGURE 21. Plot of naphthalene fluorescence intensity vs. concentration, using the experimental apparatus shown in Figure 20.

the increased complexity of operating at cryogenic temperatures should be considered first and the use of lasers to improve limits of detection in such detectors is proven.

E. Micro-Raman Spectroscopy

Etz et al.[55] at the National Bureau of Standards (NBS) have reported on a feasibility study aimed at determining the applicability of micro-Raman spectroscopy to analyses of PAH. The technique has been designed for use on single microparticles, (2 to 10 μm). The NBS-developed laser-Raman microprobe (details of which are found in Reference 55) was used for the studies. The spectra were obtained with 514.5 nm (green), 568.2 nm (yellow), and 647.1 nm (red) lines from an Ar/Kr ion laser.

In the Raman measurement, the excitation source beam which is ideally monochromatic, is focused on the sample. The radiation scattered by the sample contains weak lines which lie at frequencies both higher and lower than the excitation frequency. The frequency shifts, called Raman shifts from the excitation frequency, are characteristic of the particular molecule. The observed pattern of frequency shifts provides a means for unequivocal identification.

Initial studies on pyrene, chrysene, fluoranthene, and phenanthrene indicate that the micro-Raman spectra may be characteristically unique for all PAH molecules. Limits of detection are favorable also, on the order of 10 to 100 pg.

Further research is being directed toward the possibility of coupling this technique to a high pressure liquid chromatograph. The selectivity and sensitivity already demonstrated certainly justify this aim.

IX. CONCLUDING COMMENTS

The large and diverse number of spectroscopic techniques reported in this review deserves a proper overview. All of the reported methods can fall into two major categories: those designed as detectors for chromatographic techniques and those primarily applicable to analysis of simple mixtures. Tables 7 and 8 summarize the basic charac-

Table 7
SURVEY OF SPECTROSCOPIC TECHNIQUES FOR PAH ANALYSIS

Technique	Technical complexity[a]	Number of PAH which can be simultaneously analyzed	Detection limit for PAH (ng)	Samples studied	Ref.
Synchronous spectroscopy	4	5—8	0.1	Waste waters "Synthoil"	12—19
Room temperature phosphorimetry	5	2—4	0.05	"Synthoil"	3—11
Matrix isolation				Synthetic fuel oils	28—37
Fluorescence	8	8—10	0.01	HPLE extracts	
Infrared	9		10—100	Liquid coal fuels	
Shpol'skii effect spectroscopy	7	8—10	0.1—1.0	Air pollution sample extracts River water extracts	20—27
X-ray excited optical luminescence	7	10—15	0.1	Air particulate extracts Liquid fuels Coal solvents	47—49
Fluorescence line-narrowing spectroscopy	8	5—10	0.01	Aqueous environmental samples Model samples	50, 51
Sensitized fluorescence	1	Total fluorescence measured	Total fluorescence 0.001	Fly ash water water Air pollution sample extracts	45, 46

[a] 1 = least complex; 10 = most complex.

teristics of the techniques as well as provide information on the commercial availability of the instrument systems.

The need for a very simple method of prescreening samples to indicate the presence or lack of PAH at trace levels seems to have been met by the sensitized fluorescence method discussed earlier. The simplicity of the method should prompt all laboratories to have this technique on hand whenever needed.

With a small increase in instrumental complexity, room temperature phosphorimetry adds a further degree of selectivity to the analysis of simple mixtures of PAH. RTP is likely to find an important role in industrial on-line monitoring applications as a result of the successful research designed to automate the procedure. In many situations where the sample composition complexity is known to a certain degree of confidence the need for preseparation procedures is avoided. The recent growth in publications utilizing RTP in both pharmaceutical, industrial, and environmental applications suggests the applicability of the technique.

The simple ultraviolet and fluorescence detectors with continuum excitation sources for use with room temperature solutions have undergone a thorough modernization in an effort to increase both the sensitivity and selectivity of the detector of PAH. The various low temperature luminescence techniques have been designed to add a further refinement which results from the conversion of broad-banded fluorescence spectra of PAH at room temperature to narrow-line fluorescence spectra at cryogenic temperatures. The results have clearly indicated the success achieved towards this goal. In many cases, simple mixtures of three to five PAH can be characterized and quantified. How-

Table 8
CLASSIFICATION OF SPECTROSCOPIC INSTRUMENTATION

Analytical technique	Instrumental class[a]	Commercial availability of major instrumental components[b]
Synchronous spectroscopy	2	Spectrofluorimeter which allows the excitation and emission wavelength to be locked together (Perkin-Elmer Model 43A)
Room Temperature phosphorimetry	3	Spectrofluorimeter (Model 43A Perkin Elmer) Sample holder must be constructed (see references)
Matrix isolation (infrared and fluorescence)	3	Closed cycle helium refrigerator (Spectrim, CTI Cryogenics, Waltham, MA) Digilab FTS-20 IR Spectrometer (Digilab Inc., Cambridge, MA) High-resolution spectrofluorimeter (constructed in-lab)
Shpol'skii effect spectroscopy	3	Spectrofluorimeter (constructed in-lab) Aminco Dewar® silica cell
Fluorescence line-narrowing spectrometry	3	Liquid helium Dewar® with quartz optical windows (3-L Pope Scientific) Argon-ion laser with multiple outputs in UV region (Control 553) McPherson Model 218 Fluorescence spectrometer
X-ray excited optical luminescence	3	Spectrofluorimeter Tungsten target X-ray tube (OEG-50-T Machlett, Springdale CT)

[a] Class 1: entire system can be bought prepackaged in one instrumental unit. Class 2: analytical system can be obtained by minor modification of a prepackaged, commercially available instrument. Class 3: analytical system can be obtained by combination of two or more commercially available component parts and instruments with some laboratory modification possible.

[b] Commercial instruments which are cited were used by the authors in the review and are not necessarily the personal preference of the review authors.

ever, the increased resolution is achieved at the cost of greater instrumental complexity. The interfacing between the four low temperature methods — Shpol'skii, matrix isolation, X-ray excited optical luminescence, and fluorescence line-narrowing spectrometry — and a chromatograph must become cost effective. If not, one might be tempted to search for more highly efficient chromatographic columns which could provide complete separations and modify the present fluorescence or ultraviolet detectors with synchronous mode, derivative, and wavelength modulation capabilities. Derivative and wavelength modulation techniques can find limited applicability in assisting the resolution of interfering peaks. However, we should note the studies of Christian et al.[56-59] at the University of Washington with computer-assisted manipulation of fluorescence data. The computer-controlled methods of multicomponent analysis, such as the matrix method of rank annihilation, are far more general in scope and can be applied to any

mixture of PAH. We can envision a day when it will be routine for laboratory operators of high pressure liquid chromatographs to sit in front of a television screen and be able to enter the necessary information required to unravel and completely characterize a mixture of PAH resulting from an unresolved peak.

Finally, the goal of increased sensitivity would seem to lie in the development of laser-light excitation sources. Complete agreement on this point is reflected in the recent literature.

REFERENCES

1. Futoma, D. J., Tanaka, J., Smith, T. E., and Smith, S. R., *Crit. Rev. Anal. Chem.*, 12, 87, 1981.
2. Berlman, I. B., *Handbook of Fluorescence Spectra of Aromatic Molecules*, Academic Press, New York, 1971.
3. Vo-Dinh, T., Walden, G. L., and Winefordner, J. D., *Anal. Chem.*, 49, 1126 (1977).
4. Bower, E. L. and Winefordner, J. D., *Anal. Chim. Acta*, 102, 1 (1978).
5. Vo-Dinh, T., Bower, E. L., and Winefordner, J. D., *Talanta*, 24, 146 (1977).
6. Gammage, R. B., Vo-Dinh, T., Hawthorne, A. R., Thorngate, J. H., and Parkinson, W. W., in *Carcinogenesis, Vol. 3: Polynuclear Aromatic Hydrocarbons*, edited by Jones, P. W. and Freudenthal, R. I., Eds., Raven Press, 1978, 155.
7. Vo-Dinh, T. and Hooyman, J. R., *Anal. Chem.*, 51, 1915 (1979).
8. Vo-Dinh, T. and Gammage, R. B., *Anal. Chim. Acta*, 107, 261 (1979).
9. Vo-Dinh, T. and Gammage, R. B., in *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*, Bjørseth, A. and Dennis, A. J., Eds., Battelle Press, Columbus, Ohio, 1980.
10. Vo-Dinh, T., Gammage, R. B., and Martinez, P. R., *Anal. Chim. Acta*, 118, 313 (1980).
11. Parker, R. T., Freedlander, R. S., and Dunlap, R. B., *Anal. Chim. Acta*, 119, 189 (1980).
12. Vo-Dinh, T., *Anal. Chem.*, 50, 396 (1978).
13. Lloyd, J. B. F. and Evett, I. W., *Anal. Chem.* 49, 1710 (1977).
14. Vo-Dinh, T., Gammage, R. B., and Hawthorne, A. R., in *Polynuclear Aromatic Hydrocarbons*, Jones, P. W. and Leber, P., Eds., Ann Arbor Sci. Publ., Ann Arbor, 1979.
15. Vo-Dinh, T., Gammage, R. B., Hawthorne, A. R., and Thorngate, J. H., NBS Spec. Publ. No. 519, National Bureau of Standards, Washington, D.C. 1979, 679.
16. Vo-Dinh, T. and Gammage, R. B., *Anal. Chem.*, 50, 2054 (1978).
17. Vo-Dinh, T., Gammage, R. B., Hawthorne, A. R., and Thorngate, J. H., *Environ. Sci. Technol.*, 12, 1297 (1978).
18. Latz, H. W., Ullman, A. H., and Winefordner, J. D., *Anal. Chem.*, 50, 2148 (1978).
19. Latz, H. W., Ullman, A. H., and Winefordner, J. D., *Anal. Chem.*, 52, 191 (1980).
20. Shpol'skii, E. V., Il'ina, A. A., and Klimova, L. A., *Dokl. Akad. Nauk. U.S.S.R.*, 87, 935 (1952).
21. Kirkbright, G. F. and de Lima, C. G., *Analyst*, 99, 338 (1974).
22. Causey, B. S., Kirkbright, G. F., and de Lima, C. G., *Analyst*, 101, 367 (1976).
23. Farooq, R. and Kirkbright, G. F., *Analyst*, 101, 566 (1976).
24. Monarca, S., Causey, B. S., and Kirkbright, G. F., *Water Res.*, 13, 508 (1979).
25. Cölmsjo, A. and Stenberg, U., in *Polynuclear Aromatic Hydrocarbons*, Jones, P. W. and Leber, P., Eds., Ann Arbor Sci. Publ., Ann Arbor, 1979.
26. Cölmsjo, A. and Stenberg, U., *Anal. Chem.*, 51, 145 (1979).
27. Cölmsjo, A. and Östman, C. E., *Anal. Chem.*, 52, 2093 (1980).
28. Mamantov, G., Wehry, E. L., Kemmerer, R. R., and Hinton, E. R., *Anal. Chem.*, 49, 86 (1977).
29. Stroupe, R. C., Tokousbalides, P., Dickinson, R. B., Wehry, E., and Mamantov, G., *Anal. Chem.*, 49, 701 (1977).
30. Tokousbalides, P., Wehry, E. L., and Mamantov, G., *J. Phys. Chem.*, 81, 1769 (1977).
31. Tokousbalides, P., Hinton, E. R., Dickinson, R. B., Bilotta, P. V., Wehry, E. L., and Mamantov, G., *Anal. Chem.*, 50, 1189 (1978).
32. Wehry, E. L., Mamantov, G., Kemmerer, R. R., Stroupe, R. C., Tokousbalides, P. T., Hinton, E. R., Hembree, D. M., Dickinson, R. B., Garrison, A. A., Bilotta, P. V., and Gore, R. R., in *Carcinogenesis: Vol. 3: Polynuclear Aromatic Hydrocarbons*, Jones, P. W. and Freudenthal, R. I., Eds., Raven Press, New York, 1978.
33. Hinton, E. R., Mamantov, G., and Wehry, E. L., *Anal. Lett.*, 12(A13), 1347 (1979).
34. Dickinson, R. B. and Wehry, E. L., *Anal. Chem.*, 51, 778 (1979).
35. Wehry, E. L. and Mamantov, G., *Anal. Chem.*, 51, 643A (1979).

36. **Wehry, E. L., Mamantov, G., Hembree, D. M., and Maple, J. R.,** in *Polynuclear Aromatic Hydrocarbons — Chemistry and Biological Effects,* Bjørseth, A. and Dennis, A. J., Eds., Battelle Press, Columbus, Ohio, 1980.
37. **Maple, J. R., Wehry, E. L., and Mamantov, G.,** *Anal. Chem.,* 52, 920 (1980).
38. **O'Haver, T. C.,** in *Modern Fluorescence Spectroscopy,* E. L. Wehry, E. L., Ed., Plenum Press, New York, 1976.
39. **O'Haver, T. C.,** *Anal. Chem.,* 51, 91A (1979).
40. **Hawthorne, A. R., Thorngate, J. H., Gammage, R. B., and Vo-Dinh, T.,** NBS Spec. Publ. No. 519, National Bureau of Standards, Washington, D.C., 1979, 719.
41. **Hawthorne, A. R. and Thorngate, J. H.,** *Chem. Abstr.,* 88, 182091c (1978).
42. **Hawthorne, A. R. and Thorngate, J. H.,** *Appl. Opt.,* 17, 724 (1978).
43. **Hawthorne, A. R., Thorngate, J. H., Gammage, R. B., and Vo-Dinh, T.,** in *Polynuclear Aromatic Hydrocarbons,* Jones, P. W. and Leber, P., Eds., Ann Arbor Sci. Publ., Ann Arbor, 1979.
44. **O'Haver, T. C. and Parks, W. M.,** *Anal. Chem.,* 46, 1886 (1974).
45. **Smith, E. M. and Levins, P. L.,** Sensitized Fluorescence for the Detection of Polycyclic Aromatic Hydrocarbons, Report No. EPA-600/7-78-182 on EPA Contract No. 68-02-2150.
46. **Smith, E. M. and Levins, P. L.,** in *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects,* edited by Bjørseth, A. and Dennis, A. J., Eds., Battelle Press, Columbus, Ohio, 1980.
47. **D'Silva, A. P., Oestrich, G. J., and Fassel, V. A.,** *Anal. Chem.,* 48, 915 (1976).
48. **Woo, C. S., D'Silva, A. P., Fassel, V. A., and Oestrich, G. J.,** *Environ. Sci. Technol.,* 12, 173 (1978).
49. **Woo, C. S., D'Silva, A. P., and Fassel, V. A.,** *Anal. Chem.,* 52, 159 (1980).
50. **Brown, J. C., Edelson, M. C., and Small, G. J.,** *Anal. Chem.,* 50, 1394 (1978).
51. **Brown, J. C., Duncanson, J. A., and Small, G. J.,** *Anal. Chem.,* 52, 1711 (1980).
52. **Richardson, J. H. and Ando, M. E.,** *Anal. Chem.,* 49, 955 (1977).
53. **Richardson, J. H., George, S. M., and Ando, M. E.,** NBS Spec. Publ. No. 519, National Bureau of Standards, Washington, D. C., 1978, 691.
54. **Richardson, J. H., Larson, K. M., Haugen, G. R., Johnson, D. C., and Clarkson, J. E.,** *Anal. Chim. Acta,* 1161, 407 (1980).
55. **Etz, E. S., Wise, S. A., and Heinrich, K. F.,** NBS Spec. Publ. No. 519, National Bureau of Standards, Washington, D.C., 1978, 723.
56. **Johnson, D. W., Callis, J. B., and Christian, G. D.,** *Anal. Chem.,* 49, 747A (1977).
57. **Ho, C.-N., Christian, G. D., and Davidson, E. R.,** *Anal. Chem.,* 50, 1108 (1978).
58. **Ho, C.-N., Christian, G. D., and Davidson, E. R.,** *Anal. Chem.,* 52, 1071 (1980).
59. **Ho, C.-N., Christian, G. D., and Davidson, E. R.,** *Anal. Chem.,* 53, 92 (1981).