component appear together in the spectrum and lead to congestion. The nonlinear, four-wave mixing spectroscopy was applied to see if one could produce spectra from the individual sites without contributions from the other sites. We found that one could obtain single site spectra for the vibrational, electronic and vibronic states separately with high selectivity. There were, in fact, no contributions visible in the spectra from even the nearby sites.

The pentacene in benzoic acid doped with p-terphenyl system was studied as a model to see if one could eliminate inhomogeneous broadening. We used both parametric and nonparametric techniques to test the characteristic of each. We found that both methods could eliminate inhomogeneous broadening to the limit of our laser resolution.

This result was a surprise because the theory for the method predicts that only nonparametric methods could successfully eliminate inhomogeneous broadening. The explanation for the observation of parametric nonlinear mixing removing inhomogeneous broadening could only rest on the possibility that the character of the mixing changes as a function of the laser power because of the participation of excited state populations. A new theory was developed that treated saturation effects correctly and described excited state populations. The theory predicts intensity dependent changes in the relative intensity of the narrowed line to the non-narrowed line. It also predicts that the relative intensities are dependent upon the amount of inhomogeneous broadening. These predictions are consistent with the experimental observations.

At this point, we have succeeded in developing a new class of high resolution laser spectroscopy methods based on nonlinear mixing. The methods do not require that the sample emit light. We have applied the methods to molecular and atomic spectroscopy and are now ready to extend the methods to realistic samples and mixtures. The methods are capable of measuring both bulk concentrations and surface concentrations. Surface specific analysis is possible because nonlinear mixing processes that are based on three wave mixing are only allowed for noncentrosymmetric systems. Thus, three wave mixing will not be observed for the bulk of a material but it will be observed at the interfaces between materials. It is also important to realize that the selectivity is determined on the time scale of the lasers and is not limited by the lifetime of excited states. An environment can change while the excited state is occupied and the changes will act to

broaden the spectral line. However, if a picosecond excitation is used in the line narrowing experiment, the motion will be frozen and one can eliminate the broadening. This idea has important implications for solution spectroscopy because the motion within a solvent acts to broaden all of the lines that one would normally observe. There is, in fact, a rich field of opportunity for the development of these spectroscopies that is only being hinted at in our present work and it will be exciting to see what capabilities lie in the future.

Acknowledgment

Trace Analysis of Aromatic Compounds in Natural Samples by Shpol'skii Spectroscopy

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Introduction

Polycyclic aromatic hydrocarbons (PAH) and their alkylated derivatives are generally mutagenic or carcinogenic compounds and are common trace components of environmental samples. Besides classical analytical methodologies such as capillary gas chromatography and liquid chromatography, high resolution spectrofluorometry (HRS) at low temperature in n-alkane matrices (Shpol'skii effect) is seeing an increasing interest [1–3].

Since the first observation in the early fifties [4], this method has been extensively applied to the analysis of common PAH (e.g., pyrene, benzo(a)pyrene...), of alkylated derivatives [5,6] or heteroatom containing PAH [7,8] in various crude samples or chromatographic extracts. Results presented in this abstract demonstrate the capability of the Shpol'skii spectroscopy to quantify the PAH priority pollutants.

Experimental Requirements

Fluorescence and phosphorescence spectra of aromatic compounds usually present broad bands at room temperature, having full widths at half-maximum *(FWHM)* of about 3 nanometers. A sharpening of the luminescence spectra is observed when PAH are incorporated into an appropriate n-alkane matrix frozen at low temperature $(T \ll 77$ K). The low temperature luminescence spectra exhibit a 0-0 transition with several sharp peaks ("quasi-lines") having FWHM about 0.1 nm called multiplet structure and related to different substitution sites of the aromatic molecules in the n-alkane lattice [9].

Experimental conditions for obtaining sharp emission spectra are specific. Particularly, a remarkable matching in length of long axis and short axis of the guest (aromatic) and the host (n-alkane) molecules seems to exist [10]. When this "key and hole" rule is not respected, broad band or complicated quasilinear emission spectra are observed.

A preliminary fast freezing of solution at 77 K in liquid nitrogen is also necessary to avoid aggregate formation [11]. Another feature is also the concentration dependence of the intensity of the quasi-lines. However at low concentration (about 10^{-6} M and less), the formation of aggregates is minimized and the reproducibility of fluorescence intensity is not altered [11,12].

Quantitative Analysis of Selected PAH

The American Environmental Protection Agency (EPA) has retained about 16 PAH as priority pollutants [13]. They have been partially identified by GC-MS or HPLC. Most of these molecules exhibit well resolved emission spectra (fig. 1) in n-octane at 15 K by using a low temperature spectrofluorometer previously described [14]. Perdeuterated PAH (deuterated pyrene and benzo(a)pyrene) have been used as internal standards and have been added to the total organic extract. These compounds exhibit a similar Shpol'skii emission spectra to that of the respective parent compound but with a spectral line shift of about 1 nm.

Most of the EPA PAH could easily be identified and quantified by this technique. Low molecular weight aromatic compounds (acenaphthene, acenaphthalene, fluorene, phenanthrene and anthracene) were difficult to detect due to their low fluorescence quantum yield. To evaluate the accu-

racy of this method, we have analyzed several organic extracts (diesel particulate, air particulate) used for intercalibration exercises organized by the National Bureau of Standards (Gaithersburg, MD USA). The results (imprecision less than 15%) obtained by Shpol'skii spectroscopy have been compared with values obtained by other analytical methodologies (HPLC coupled with fluorescence detection, GC coupled to mass spectrometry). As presented in figure 2, good agreement is observed among the three analytical techniques, demonstrating the reliability of Shpol'skii spectroscopy for quantitative analysis.

Conclusion

Partial results presented here show the analytical capability of high resolution spectrofluorometry in Shpol'skii matrices for the determination of different priority pollutant aromatic compounds in the total organic extract of natural samples.
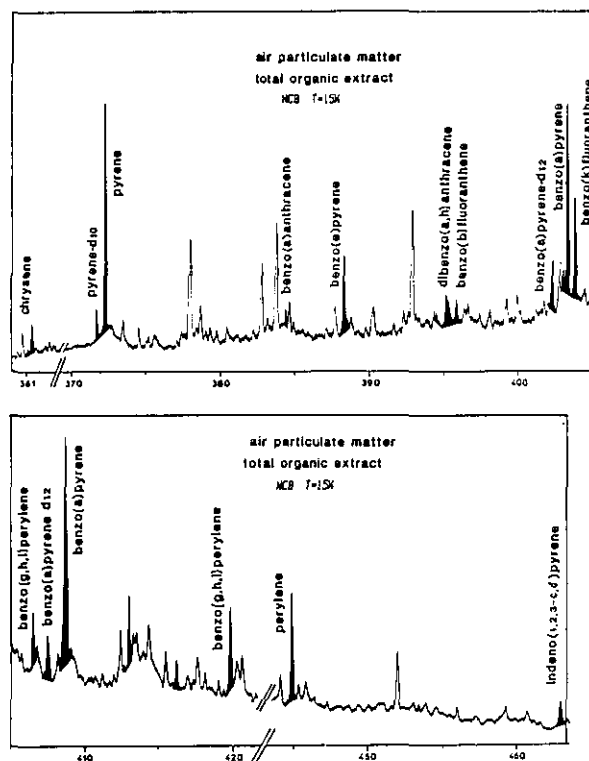

Figure 1. Fluorescence emission spectra of the organic air particulate extract in n-octane at 15 K. Deuterated pyrene and benzo(a)pyrene have been added as internal standards. Excitation wavelengths centered respectively at 275 nm (top) and at 290 nm (bottom).
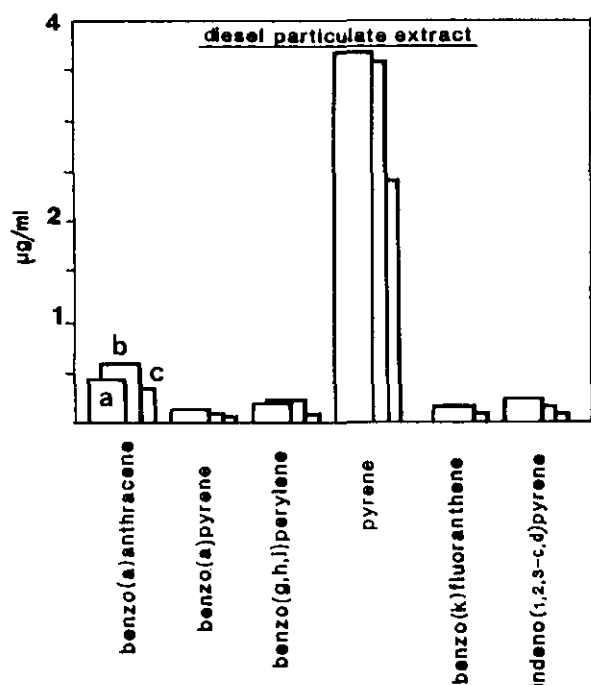
Accuracy in Trace Analysis

Figure 2. Comparison of the concentration values obtained on a diesel particulate extract for PAH determined by several analytical techniques (International Round Robin organized by the National Bureau of Standards, Gaithersburg, MD, USA): a) Capillary gas chromatography coupled to mass spectrometry (NBS values, ref. 15). b) Liquid chromatography coupled to spectrofluorometry (ref. 15). c) Shpol'skii spectroscopy values.

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Sodium Taurocholate Micelles in Fluorometrix Analysis

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Sodium taurocholate ($3\alpha,7\alpha,12\alpha$-trihydroxy-5β-cholanoyl taurine, sodium salt), NaTC, is a micelle-forming bile salt. The three hydroxy groups on the cholic acid part of the molecule play an important role in the micellar characteristics of NaTC, and, along with the taurine group, serve to solubilize NaTC in aqueous solutions [1]. The most commonly observed micellar form has an aggregation number of four and can bind other molecules in the relatively hydrophobic interior, usually with a 1:1 stoichiometry [2]. The formation of secondary micelles has not been observed. Insoluble biological lipids can be solubilized by comicellization with NaTC [3] and NaTC can also form mixed micelles with detergent molecules [2]. The micellar properties of NaTC are relatively insensitive to experimental conditions. The aggregation number and critical micelle concentration (CMC) show very little dependence on pH in the range of 1.6–10 [1], on counterion concentration, and on temperature in the range of 10–60 °C [3]. A CMC of 3 mM was found from the shift in the absorption wavelength maximum of Rhodamine 6G [3].

We have used fluorescence probe molecules to study NaTC micelles alone and in NaTC-detergent mixed micellar solutions. Results were compared with those obtained for individual detergent micellar solutions, including SDS, Triton X-100 (reduced form) and CTAC. Pyrene was used as a probe of the micellar binding site polarity, and perylene was used in fluorescence lifetime and polarization studies. Nonradiative energy transfer