

separated from F 80 and no other T4CDF isomer coelutes with 2,3,7,8-T4CDF.

The question of whether or not this column combination allows the separation and determination of the other congeners that cannot be separated by isomer-specific capillaries was also investigated. Of special interest was the determination of the 2,3,7,8-class congeners with this combination. We observed difficulties in the determination of several congeners of the 2,3,7,8-class through interferences with other congeners. 1,2,3,6,9-P5CDD (D 53) coelutes with 1,2,3,7,8-P5CDD (D 54) if we use more than 4.5 m of DB 17. There exist no difficulties in the determination of the three 2,3,7,8-substituted H6CDD's (D 66, D 67, D 70), the two 2,3,7,8-substituted H7CDF's (F 131, F 134), and 1,2,3,4,6,7,8-H7CDD (D 73). 1,2,3,7,8-P5CDF (F 94) still coelutes with 1,2,3,4,8-P5CDF (F 89) as it does on isomer-specific capillaries and an interference from 1,2,3,4,6-P5CDF (F 87) is observed, depending on the additional length of DB 17. 1,2,3,4,7,8-H6CDF (F 118) still cannot be separated from 1,2,3,4,7,9-H6CDF (F 119) and 1,2,3,4,8,9-H6CDF (F 120) interferes with 2,3,4,6,7,8-H6CDF (F 130), depending on the additional length of DB 17. For experimental details, see ref 13.

The combination of the capillaries SP 2331 + DB 17 (30/60 m + 12.5 m) is able to separate 2,3,7,8-T4CDF (F 83) from 2,3,4,8-T4CDF (F 80) without interferences from other T4CDF congeners. This is of special importance for samples with great differences in the 2,3,7,8-/2,3,4,8-T4CDF ratio, for instance technical mixtures of PCB's. The durability of the column combination is about the same as SP 2331 alone. One advantage is the possibility of rinsing the DB 17 column with solvents to remove dirt from the column head.

On basic alumina 2,3,7,8-T4CDF (F 83) can be separated from all other T4CDF isomers. This separation is sometimes impossible to perform cleanly because of matrix effects on the fractionation. Depending on the type of matrix an elution of some T4CDF congeners in the second alumina fraction or a partial elution of 2,3,7,8-T4CDF in the first fraction is observed. Experience shows that its use in the accurate analytical routine is difficult and requires the use of isotopically labeled 2,3,7,8-T4CDF for the control of the fractionation and accurate quantitation. If both methods are combined the

accurate quantitation of 2,3,7,8-T4CDF can be done.

## ACKNOWLEDGMENT

We thank J. Andersson for critically reading the manuscript.

**Registry No.** 2,3,7,8-T4CDF, 51207-31-9; 1,2,3,4-T4CDF, 24478-72-6; 1,2,3,6-T4CDF, 83704-21-6; 1,2,3,7-T4CDF, 83704-22-7; 1,2,3,8-T4CDF, 62615-08-1; 1,2,3,9-T4CDF, 83704-23-8; 1,2,4,6-T4CDF, 71998-73-7; 1,2,4,7-T4CDF, 83719-40-8; 1,2,4,8-T4CDF, 64126-87-0; 1,2,6,7-T4CDF, 83704-25-0; 1,2,6,8-T4CDF, 83710-07-0; 1,2,6,9-T4CDF, 70648-18-9; 1,2,7,8-T4CDF, 58802-20-3; 1,2,8,9-T4CDF, 70648-22-5; 1,3,4,6-T4CDF, 83704-27-2; 1,3,4,8-T4CDF, 92341-04-3; 1,3,6,7-T4CDF, 57117-36-9; 1,3,6,8-T4CDF, 71998-72-6; 1,3,6,9-T4CDF, 83690-98-6; 1,3,7,8-T4CDF, 57117-35-8; 1,3,7,9-T4CDF, 64560-17-4; 1,4,6,7-T4CDF, 66794-59-0; 1,4,6,8-T4CDF, 82911-58-8; 1,4,7,8-T4CDF, 83704-29-4; 1,6,7,8-T4CDF, 83704-33-0; 2,3,4,6-T4CDF, 83704-30-7; 2,3,4,7-T4CDF, 83704-31-8; 2,3,4,8-T4CDF, 83704-32-9; 2,3,6,7-T4CDF, 57117-39-2; 2,3,6,8-T4CDF, 57117-37-0; 2,4,6,7-T4CDF, 57117-38-1; 2,4,6,8-T4CDF, 58802-19-0; 3,4,6,7-T4CDF, 57117-40-5; Chlophen A 60, 11096-99-4.

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RECEIVED for review January 29, 1987. Accepted June 9, 1987.

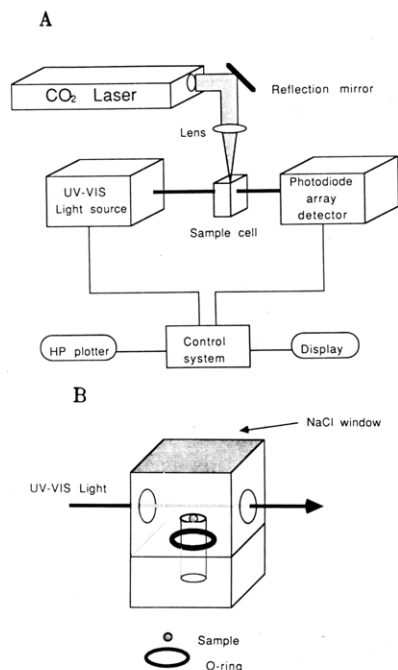
## Ultraviolet-Visible Absorption Spectra of Biological Molecules in the Gas Phase Using Pulsed Laser-Induced Volatilization Enhancement in a Diode Array Spectrophotometer

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Numerous studies have appeared recently in the analytical literature of various forms of high-powered, monochromatic laser excitation spectroscopy for gas-phase analysis of molecules. Such techniques include laser-induced fluorescence (1-3), laser-induced dispersed fluorescence (4), laser absorption (5), and laser multiphoton ionization (6, 7) experiments. These techniques are often combined with the supersonic jet technique in order to produce rotationally cold molecules. At room temperature the spectra of most large polyatomic molecules consist of a broad unresolvable congestion of rotational lines so that one cannot take advantage of the monochromatic nature of the laser light for exact identification. However, the sharp spectral features (<0.2 Å) obtained under jet ex-

pansion conditions makes this an excellent technique for enhancing selectivity for chemical analysis. These experiments though require some prior knowledge of the general gas-phase absorption contours of the molecules under study. For reasonably volatile compounds or molecules that are stable upon heating, UV-vis absorption spectrophotometers can be used to obtain the broad gas-phase contours. It is important to know not only the general region in which the molecule absorbs but especially the onset of absorption, since this will generally be near the origin band. This is important for analysis in jet experiments where identification often depends on the position of several sharp absorption bands near the origin. The approximate position of these bands can often



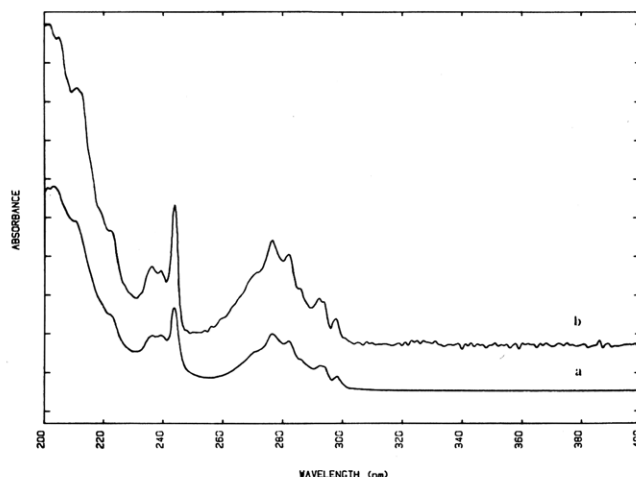
**Figure 1.** (A) Schematic diagram of the LIVE/UV-vis spectroscopic setup. (B) Sample holder and containment cell for laser-induced volatilization.

be guessed within several nanometers based upon the UV-vis gas-phase absorption taken in a standard spectrophotometer under thermal conditions.

This method has worked well for compounds with sufficient vapor pressure upon heating that do not undergo significant decomposition. However, the gas-phase absorption of most nonvolatile and thermally labile molecules is unknown. Since these include significant classes of biological and pharmaceutical compounds, a method of obtaining the absorption spectra of these molecules is critical if these methods are to be systematically extended to these molecules. Although the liquid-phase spectra of these molecules provide a general absorption contour solvent shifts may be significant depending on the solvent used. Considering the bandwidth of the laser excitation ( $1\text{ cm}^{-1}$ ), even a solvent shift as small as 5–10 nm may be considered as significant. In this short aid, we therefore present a method of obtaining gas-phase absorption spectra of small labile molecules of biological significance by using a combination of pulsed laser-induced volatilization enhancement (LIVE) and a diode array spectrophotometer.

## EXPERIMENTAL SECTION

The experimental setup is shown in Figure 1. A pulsed CO<sub>2</sub> laser ( $10.6\text{ }\mu\text{m}$ ) is used to induce desorption of neutral species in the light path of commercial UV-vis spectrophotometer (Hewlett-Packard 8450A) with a photodiode array detector. This device allows us to record the whole UV-vis absorption spectrum of the desorbed material within 1 s of the desorption event. The CO<sub>2</sub> laser (Quanta-Ray EXC-1) has an  $\sim 80\text{-ns}$  output spike pulse followed by an  $\sim 1\text{-}\mu\text{s}$  decay pulse. The laser beam is focused by using a Ge lens (focal length  $\sim 175\text{ mm}$ ) to an  $\sim 1\text{-mm}$  spot on the Macor machinable ceramic surface on which the sample was placed, and vaporization was induced. The power density on the surface is estimated to be  $\sim 10^7\text{ W/cm}^2$  in the samples studied herein, although lower power can be used for molecules with lower melting points. In each case the vaporization was performed under atmospheric pressure conditions. The position of the ceramic surface and laser beam was optimized so that as much of the desorbed plume intersected the UV-vis spectrophotometer light beam as possible. In order to concentrate the plume in the light-beam path for an extended period of time, a containment cell was placed above the ceramic desorption surface. This consists of a simple polystyrene cell with two open holes for the UV-vis



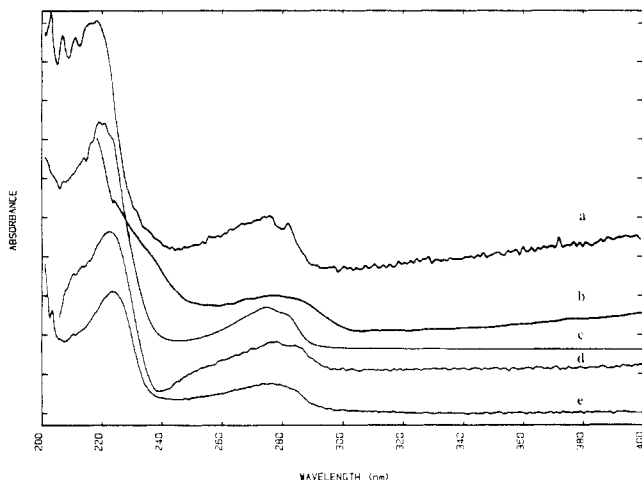
**Figure 2.** Comparison between the gas-phase UV spectrum of dibenzofuran obtained by (a) heating to  $127\text{ }^{\circ}\text{C}$  and (b) laser-induced volatilization.

light beam to pass through and a NaCl window on top in order to allow the CO<sub>2</sub> laser beam to be transmitted to the ceramic surface.

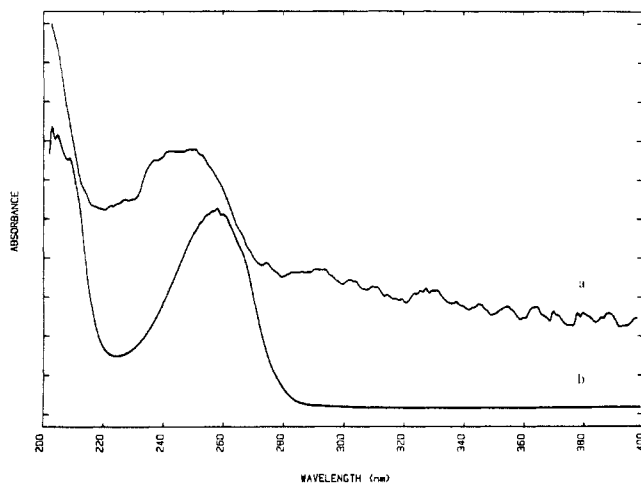
This experiment is possible due to the ability of the diode array detector to acquire a complete spectrum every second. The typical sample, which generally consists of rather thick samples weighing  $\sim 1\text{ mg}$ , on the surface is completely desorbed within  $\sim 30\text{ s}$  at the power levels used herein, although in the present configuration only a fraction of these actually traverse the beam path and contribute to the spectrum. However, spectra have been obtained with an initial sample of  $<100\text{ }\mu\text{g}$ . The molecules studied have absorptions in the near-UV so that the spectrophotometer was programmed to take data in the 200–400-nm region. The CO<sub>2</sub> laser power and pulse repetition rate ( $\sim 10\text{--}100\text{ Hz}$ ) have been optimized in each case to produce the best spectrum. A Nd:YAG laser ( $1.06\text{ }\mu\text{m}$ ) could also be used for laser-induced volatilization. We have found in our earlier work though (8) that the advantage of the CO<sub>2</sub> laser is that lower energy photons are less likely to cause subsequent multiphoton absorption/fragmentation/ionization that might interfere with the spectrum. In addition, scattered  $10\text{-}\mu\text{m}$  radiation from the CO<sub>2</sub> laser is not detected by the diode array device and thus background noise is minimized from this source, whereas the spectrophotometer may respond to scattered near-IR or visible light from the Nd:YAG laser or its second harmonic.

## RESULTS AND DISCUSSION

The purpose of this work is to provide a means of obtaining gas-phase UV-vis absorption spectra of nonvolatile molecules as a guide for investigators involved in studying the spectroscopy of these species by various laser-based techniques. The first question that must be addressed is whether the spectra obtained by laser desorption is the same as that obtained by heating the material in a gas-phase sample cell. In Figure 2a we show a spectrum of dibenzofuran obtained by heating the sample to  $127\text{ }^{\circ}\text{C}$  and in Figure 2b a spectrum obtained by laser-induced volatilization at room temperature. The spectra are essentially identical, as was the case for other molecules studied whose spectra could be obtained by both methods. The reasons why they might be different could be due to excessively hot molecules present or a significant number of ions produced by the vaporization process. The desorption is performed at atmospheric pressure in air so that the large number of collisions probably relaxes any initially formed hot molecules back to thermal conditions. In addition, if ions were found in a significant quantity one would expect to observe a strong spectral peak in the green region of the spectrum since it has been shown that the ionic absorption in these aromatic species is shifted toward the visible (9). No extra bands due to ion production are observed. In previous



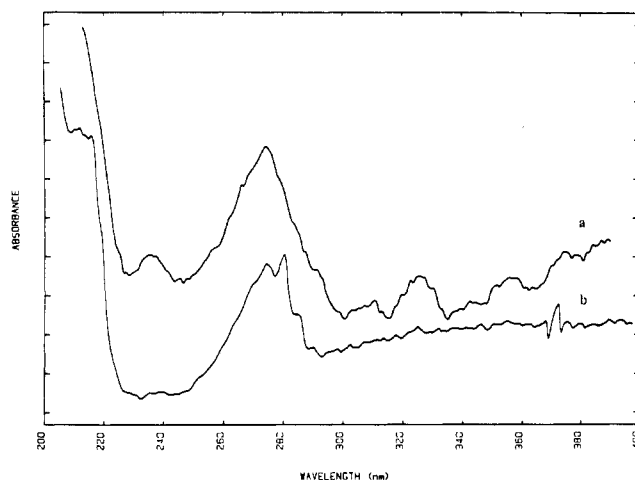
**Figure 3.** UV spectra of tyrosine (a) in gas-phase by laser-induced volatilization and in various solvents in the liquid phase including (b) cyclohexane, (c) water, (d) ethanol, and (e) methanol.



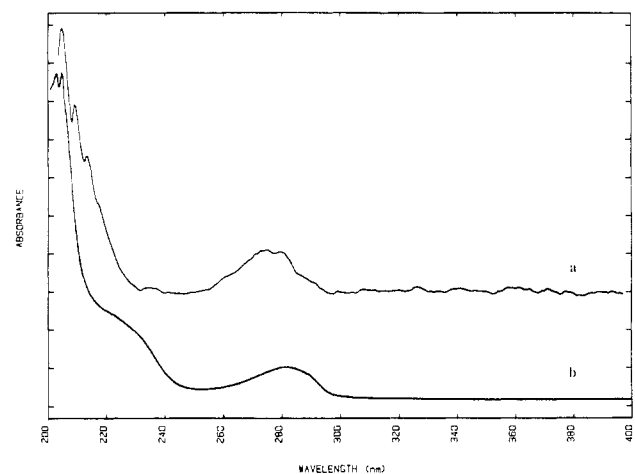
**Figure 4.** UV spectra of adenine in (a) gas phase obtained by laser-induced volatilization and (b) methanol solvent.

experiments under both vacuum (8) and atmospheric pressure conditions (10) almost no ion production was observed under the desorption conditions used.

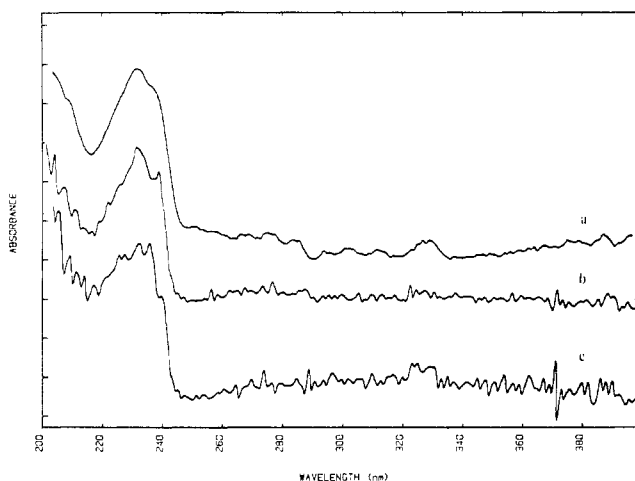
The next problem examined is whether this method will produce spectra for molecules with very high melting points or that are thermally labile. One example shown in Figure 3 is tyrosine (mp  $\sim 325^\circ\text{C}$  (dec)), where a gas-phase spectrum produced by laser desorption is compared to spectra obtained in various solvents. The spectra in solution are generally slightly shifted by several nanometers compared to the gas-phase spectrum. In addition, more vibrational structure can be observed in the LIVE gas-phase-produced spectrum than in the liquid phase. The shifts in solution generally appear at longer wavelengths as expected (11) in a  $\pi\text{-}\pi^*$  transition. We have observed similar results for other species such as tryptophan where the liquid-phase spectra in water and methanol are shifted at least 3 and 5 nm, respectively, to longer wavelengths than the laser-induced gas-phase spectrum. In Figure 4 are shown spectra of adenine (mp  $> 360^\circ\text{C}$ ) in the gas phase and in methanol. In this case a strong displacement of the liquid-phase spectrum to longer wavelengths is observed. In Figures 5 and 6 are demonstrated gas-phase UV-vis absorption spectra of several thermally labile compounds volatilized by laser desorption. These include Dopa, dopamine, and norepinephrine, which rapidly decompose upon heating to form a tarlike polymer. However, gas-phase spectra can be obtained by using the LIVE method, which resemble the



**Figure 5.** Gas-phase UV spectra of (a) dopamine and (b) norepinephrine obtained by LIVE technique.



**Figure 6.** UV spectra of Dopa in (a) the gas phase obtained by laser-induced volatilization and (b) methanol solvent.



**Figure 7.** Gas-phase UV spectra of dipeptides obtained by LIVE technique: (a) phenylalanyltirosine, (b) phenylalanylleucine, and (c) tyrosylglycine.

spectra obtained when these molecules are dissolved in various solvents, although the liquid spectra are shifted several nanometers to longer wavelengths as demonstrated in Figure 6. It should be noted that norepinephrine was purchased in the hydrochloride form; however, previous LD experiments under vacuum (8) and at atmospheric pressure (10) show that neutral norepinephrine desorbs with the detachment of the

HCl group. This was generally found to be true of compounds prepared in the HCl form (8, 10).

One last example of the utility of the laser-induced vaporization method is shown for the case of several dipeptides. In Figure 7 are shown in the gas-phase spectra of tyrosine-glycine, phenylalanine-tyrosine, and phenylalanine-leucine performed by laser-induced vaporization. The basic tyrosine spectrum has its origin around 282 nm; however, the tyrosine aromatic ring  $\pi$ - $\pi^*$  absorption is strongly shifted to the UV by other amino acids, which appear to act as electron-withdrawing groups in this case (12). Thus strong absorption bands are observed around 220-240 nm for these peptides, but only a very weak absorption is present at  $\sim$ 280 nm.

In conclusion, the LIVE technique can be used to obtain gas-phase UV-vis absorption of thermally labile molecules in a diode array spectrophotometer. The absorption contours are similar to those obtained in liquid solvents except they are shifted by several nanometers or in some cases 10-20 nm and the gas-phase spectra exhibit more vibrational structure. This technique is useful for obtaining spectral contours for spectroscopic purposes; however, in its present form is not useful for sensitivity or quantitation results.

**Registry No.** Dopa, 59-92-7; dibenzofuran, 132-64-9; L-tyrosine, 60-18-4; dopamine, 51-61-6; norepinephrine, 51-41-2; phenylalanyltyrosine, 17355-18-9; adenine, 73-24-5; phenylalanylleucine, 3303-55-7; tyrosylglycine, 673-08-5.

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RECEIVED for review March 2, 1987. Accepted July 9, 1987. We gratefully acknowledge support of this work under NSF Grant CHE-8419383. David M. Lubman is a Sloan Foundation Research Fellow.

## Measurement of Vapor Deposition and Extraction Recovery of Polycyclic Aromatic Hydrocarbons Adsorbed on Particulate Solids

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Polycyclic aromatic hydrocarbons (PAHs), released (in the vapor phase) into the atmosphere via combustion of fossil fuels, are believed to adsorb on atmospheric particulate surfaces (1, 2). The chemical behavior of an adsorbed PAH is strongly dependent on the characteristics of the adsorbent (3-5).

Studies of chemical transformations of adsorbed PAHs usually require that the PAHs and/or their transformation products be extracted from the adsorbent and then analyzed by conventional spectrometric or chromatographic methods (3-5). Some adsorbents (especially coal ashes and other carbonaceous substrates) retain PAHs with such avidity that recoveries by conventional solvent (or even supercritical fluid) extractions are much less than 100% (6-11).

Measurement of extraction recovery is straightforward when a PAH is adsorbed onto a particular solid from a liquid solution but is less facile when the adsorbate is deposited onto the adsorbent from the vapor phase. (For example, radiotracer techniques (6) are not readily applicable with adequate safety to vapor-deposition experiments.) Herein we describe techniques for measuring the quantity of organic adsorbate vapor that deposits on a particulate solid and determining the subsequent recovery of that adsorbate by extraction. The principal component of the apparatus is a gas-chromatographic flame ionization detector (FID), used to measure the mass flow rate of PAH to which the adsorbent is exposed. Pyrene is used as an example adsorbent; the chemical reactivity of pyrene adsorbed on particulate solids has been studied extensively (3).

#### EXPERIMENTAL SECTION

**Apparatus and Vapor-Deposition Technique.** Deposition of a PAH is performed by passing a stream of PAH vapor through

a bed of adsorbent at elevated temperature. The apparatus is shown schematically in Figure 1. The PAH vapor is produced in a diffusion cell (12) placed in an oven ("oven 1") held at a temperature slightly below the melting point of the PAH. The vapor is swept from the diffusion cell by a stream of N<sub>2</sub> at a constant flow rate (typically 40 mL/min) and downstream pressure drop (7.3 kPa) maintained by a flow controller (Varian 57-000285-00). For pyrene, the mass flow rate of vapor emanating from the diffusion cell can be varied over the range 50-400 g/h by altering the diffusion-cell temperature over the range 383-417 K.

The PAH/N<sub>2</sub> stream is then directed into a second oven ("oven 2" in Figure 1), which is maintained at a slightly higher temperature than oven 1. Oven 2 contains two valves (Valco V-4-HT), each of which can channel the PAH/N<sub>2</sub> stream directly from valve inlet to outlet (dashed lines, Figure 1) or divert the vapor stream through an alternate loop (solid lines). With valve 1 in deposit mode, the PAH/N<sub>2</sub> stream passes through a cell (12) containing a bed of adsorbent. With valve 1 in bypass mode, the cell is circumvented. Similarly, with valve 2 in collect mode, the vapor stream is routed to a coil of tubing immersed in a cold bath; with Valve 2 in detect mode, the vapor stream proceeds directly to an FID (Gow-Mac 12-800).

To compensate for changes in pressure drop as the route of the vapor stream is altered, pressure regulators 1 and 2 (Nupro SS-2SG) are adjusted whenever valve 1 or 2 is switched, to maintain a constant gauge pressure.

Two types of pyrene vapor deposition experiments were performed. In the first, 1-g samples of adsorbent were exposed for 1 h to a pyrene mass flow rate of 60  $\mu$ g/h (hence, the maximum quantity of pyrene that could be deposited on any adsorbent was 60  $\mu$ g). A second procedure consisted of depositing pyrene on an adsorbent until its adsorptive capacity was "saturated" (i.e., further exposure to pyrene vapor produced no detectable adsorption). These experiments are termed "60- $\mu$ g" and "capacity" depositions, respectively.

**Quantification of Vapor Deposition.** To determine the quantity of PAH that adsorbs on the substrate, the mass flow rate of PAH before ( $M_d$ ) and after ( $M_a$ ) the cell is measured.

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