Factors Affecting Quantitative Analysis in Laser Desorption/Laser Ionization Mass Spectrometry

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Microprobe laser desorption/laser ionization mass spectrometry ($\mu L^2 MS$) is a sensitive and selective technique that has proven useful in the qualitative and semiquantitative detection of trace organic compounds, particularly polycyclic aromatic hydrocarbons (PAHs). Recent efforts have focused on developing $\mu L^2 MS$ as a quantitative method, often by measuring the ratio of signal strength of an analyte to an internal standard. Here, we present evidence of factors that affect these ratios and thus create uncertainty and irreproducibility in quantification. The power and wavelength of the desorption laser, the delay time between the desorption and ionization steps, the power of the ionization laser, and the ionization laser alignment are all shown to change PAH ratios, in some cases by up to a factor of 24. Although changes in the desorption laser parameters and the delay time cause the largest effects, the ionization laser power and alignment are the most difficult parameters to control and thus provide the most practical limitations for quantitative $\mu L^2 MS$. Variation in ratios is seen in both synthetic poly(vinyl chloride) membranes and in "real-life" samples of Murchison meteorite powder. Ratios between similar PAHs vary less than those between PAHs that differ greatly in mass and structure. This finding indicates that multiple internal standards may be needed for quantification of samples containing diverse PAHs.

Microprobe laser desorption/laser ionization mass spectrometry ($\mu L^2 MS$) is a relatively new analytical technique that has proved useful in the sensitive and selective detection of specific organic components of complex mixtures. $\mu L^2 MS$ has been most widely used to detect polycyclic aromatic hydrocarbons (PAHs) and other aromatic compounds in a variety of samples, including ancient terrestrial rocks,[1] sediments and soils,[2,3] meteorites,[4,5] atmospheric aerosols,[6] agricultural samples,[7,8] polymers,[9] and natural water samples.[10] Most $\mu L^2 MS$ studies have been qualitative or semiquantitative in nature, in which this method is used to confirm the presence or absence of compounds or to spatially map the distribution of aromatics across a surface. Recently, however, several studies have claimed to use $\mu L^2 MS$ for quantitative analysis of analyte concentrations.[7,10–15] These reports have caused us to examine critically the use of $\mu L^2 MS$ for this purpose.

$\mu L^2 MS$ is a two-step process that combines laser desorption from a solid sample with resonance-enhanced multiphoton ionization (REMPI), in which the resulting ions are detected by time-of-flight mass spectrometry. The desorption and ionization steps are separated both spatially and temporally, which allows individual optimization of each step. Desorption is usually carried out by a pulsed infrared (IR) laser (although ultraviolet (UV) light has also been used[2]), which allows neutral, intact molecules to be ablated or released from a sample's surface.[16–18] REMPI is a selective and soft means of ionization that produces minimal fragmentation and ionizes only those molecules with appropriate transitions, making it ideal for analysis of trace compounds in complex natural samples.[18,19] $\mu L^2 MS$ requires almost no sample preparation, and the analysis of a sample is rapid, requiring only minutes. A complete mass spectrum is obtained for each desorption shot, which permits spatially resolved mapping of a sample surface by scanning the location of the laser desorption spot.[5] Detection limits in the attomole range can be achieved for polycyclic aromatic hydrocarbons (PAHs), lower than many conventional analytical techniques.[20,21]

(1) Mahajan, T. B.; Plows, F. L.; Gillette, J. S.; Zare, R. N.; Logan, G. A. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 975−987.

(2) Fye, J. L.; Nelson, H. H.; Mowery, R. L.; Baronavski, A. P.; Callahan, J. H. *Anal. Chem.* **2002**, *74*, 3019−3029.

(3) Gillette, J. S.; Luthy, R. G.; Clemett, S. J.; Zare, R. N. *Environ. Sci. Technol.* **1999**, *33*, 1185−1192.

(4) Clemett, S. J.; Maechling, C. R.; Zare, R. N.; Alexander, C. M. O. D. *Lunar Planet. Sci. Conf.* **1992**, *XXIII*, 233−234.

(5) Plows, F. L.; Elsila, J. E.; Zare, R. N.; Buseck, P. R. *Geochim. Cosmochim. Acta* **2003**, *67*, 1429−1436.

(6) Haefliger, O. P.; Bucheli, T. D.; Zenobi, R. *Environ. Sci. Technol.* **2000**, *34*, 2178−2183.

(7) Orea, J. M.; Montero, C.; Jimenez, J. B.; Gonzalez Ureña, A. *Anal. Chem.* **2001**, *73*, 5921−5929.

(8) Orea, J. M.; Bescos, B.; Montero, C.; Gonzalez Ureña, A. *Anal. Chem.* **1998**, *70*, 491−497.

(9) Zhan, Q.; Zenobi, R.; Wright, S. J.; Langridge-Smith, P. R. R. *Macromolecules* **1996**, *29*, 7865−7871.

(10) Bucheli, T. D.; Haefliger, O. P.; Dietiker, R., Jr.; Zenobi, R. *Anal. Chem.* **2000**, *72*, 3671−3677.

(11) Weickhardt, C.; Tonnies, K.; Globig, D. *Anal. Chem.* **2002**, *74*, 4861−4867.

(12) Kalberer, M.; Morrical, B. D.; Sax, M.; Zenobi, R. *Anal. Chem.* **2002**, *74*, 3492−3497.

(13) Haefliger, O. P.; Zenobi, R. *Rev. Sci. Instrum.* **1998**, *69*, 1828−1832.

(14) Emmenegger, C.; Kalberer, M.; Morrical, B.; Zenobi, R. *Anal. Chem.* **2003**, *75*, 4508−4513.

(15) Hankin, S. M.; John, P.; Simpson, A. W.; Smith, G. P. *Anal. Chem.* **1996**, *68*, 3238−3243.

(16) Maechling, C. R.; Clemett, S. J.; Engelke, F.;.Zare, R. N. *J. Chem. Phys.* **1996**, *104*, 8768−8776.

(17) Zenobi, R. *Chimia* **1994**, *48*, 64−71.

(18) Lubman, D. M. *Lasers and Mass Spectrometry*; Oxford University Press: New York, 1990.

(19) Boesl, U. *J. Mass Spectrom.* **2000**, *35*, 289−304.

The detection of PAHs, compounds consisting of two or more fused benzene rings, is of considerable interest both because of their natural occurrence in extraterrestrial and terrestrial samples[22-24] and because of their carcinogenic and toxic properties. It is estimated that up to 20% of all galactic carbon is contained in PAHs, making the analysis of PAH distribution in extraterrestrial samples an important part of understanding the galactic carbon cycle.[25] Closer to home, PAHs are prevalent and dangerous pollutants, emitted by a variety of combustion sources, and they contribute to air, soil, and water pollution.[26-29] Analytical techniques capable of quantifying the amount of PAHs in natural samples are essential for understanding, controlling, and remediating environmental contamination.

Recent attempts to develop quantitative μL^{2}MS have shown promising results. Quantification with μL^{2}MS, however, faces some inherent obstacles. The infrared laser desorption used in μL^{2}MS is primarily an interaction between the IR laser and the solid substrate that produces rapid heating causing thermal desorption of adsorbates.[16,17] The kinetic energy profile of the desorbed molecules depends on such factors as laser pulse energy, laser wavelength, and nature of the substrate.[30-32] The kinetic energy profile, in turn, influences the optimal delay time between the desorption and ionization steps of μL^{2}MS. Therefore, a change in desorption conditions can change the amount of signal detected for a given species.

The ionization step also poses some problems for quantitative analysis. The peak area measured for a species depends not only on the amount of that species desorbed from the surface but also on its photoionization cross section. This cross section is a function of both the UV absorption of a species and its ionization efficiency and thus differs for each species, although the cross sections for alkylated PAHs appear to be similar to those of their parent PAHs.[33] It is therefore not possible in general to use directly the ratio of two peak areas as a measure of the ratio of the concentrations of two species. Studies have, however, used a calibration curve to determine the relative instrument responses of two species, leading to estimation of concentration by use of an internal standard.[11,12,14,15]

Several approaches have been used in recent efforts toward quantitative μL^{2}MS. Good precision, accuracy, and reproducibility have been shown in the analysis of single analytes in a sample.[7,8] In these cases, creation of a calibration curve correlating signal intensity with analyte concentration has been effective. The method of standard additions, in which known quantities of the analyte are added to several identical samples, has also been shown to be effective for quantitation of a single analyte.[7] Quantitative μL^{2}MS studies, however, almost always attempt to quantify several analytes at once. Furthermore, this work is often aimed at analyzing field samples, including soils, waters, and aerosols, that may be limited in quantity.[10-12,14] In such cases, the method of standard additions becomes impractical. As a result, many quantitative μL^{2}MS studies have chosen to quantify by comparison of the analyte signal strength to that of an internal standard, relying on this ratio to correlate reproducibly with the respective concentrations of the compounds.

Fluctuations in the intensity of the desorption and the ionization lasers as well as variations in sample composition and morphology within a sample contribute to the difficulties encountered in quantitative μL^{2}MS. Standard deviations for the signal from a single species measured repeatedly from the same sample are on the order of 5−20%.[6-8,10] Variations in PAH ratios, such as the ratio of an analyte of interest to an internal standard, can be much more, with standard deviations from 10 to 36% across a sample being reported.[11,14] Although the effects of various μL^{2}MS parameters on the signal strength of single species has been investigated,[2,7,8,10] there is little work on how operational parameters and fluctuations might differentially affect different PAHs.[15] Understanding this variation is increasingly important as attempts to use quantitative μL^{2}MS with internal standards become more widespread. In this work, we have examined how changes and fluctuations in operating parameters such as desorption laser power and wavelength, ionization laser power, delay time between desorption and ionization, substrate type, and alignment of the ionization laser affect the detected signal strength for a variety of PAHs. We have focused on how these parameters change the measured ratios between PAHs, rather than on the changes in individual PAHs, to understand better the ability to use internal standards for quantitative μL^{2}MS studies. We discuss how these factors impact efforts to make μL^{2}MS measurements quantitative.

EXPERIMENTAL SECTION

μL^{2}MS System. The home-built μL^{2}MS apparatus used in this investigation has been described in detail elsewhere.[20] Briefly, the sample to be analyzed is introduced into the vacuum chamber of the apparatus, which is evacuated to ~2×10^{-8} Torr. A pulsed IR laser beam is focused through a microscope objective onto the sample surface, creating a plume of desorbed neutral molecules. Care is taken to adjust the power of the IR pulse to avoid plasma formation. The system has two pulsed IR desorption lasers: (1) a CO$_2$ laser, with a wavelength of 10.6 μm, focused to a 40-μm spot by the microscope objective (Alltech AL853 CO$_2$ laser, Alltech, Lubeck, Germany); (2) an Er:YAG laser (2.94 μm, Big Sky Laser 571 A, Big Sky Lasers, Boseman, MT), which is focused to ~10 μm. The difference in spot sizes and wavelengths between the two IR lasers can cause a difference in desorption efficiency of a given sample. Following desorption, the fourth harmonic of a pulsed Nd:YAG laser (266 nm; Spectra Physics DCR11, Spectra-

(20) Clemett, S. J.; Zare, R. N. In *Molecules in Astrophysics: Probes and Processes*; Dishoeck, E. F. v., Ed.; Kluwer Academic Publishers: Leiden, Netherlands, 1997; pp 305−320.

(21) Zhan, Q.; Voumard, P.; Zenobi, R. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 119−127.

(22) McKay, D. S.; Gibson, E. K.; Thomas-Keprta, K. L.; Vali, H.; Romanek, C. S.; Clemett, S. J.; Chillier, X. D. F.; Maechling, C. R.; Zare, R. N. *Science* **1996**, *273*, 924−930.

(23) Basile, B. P.; Middleditch, B. S.; Oro, J. *Org. Geochem.* **1984**, *5*, 211−216.

(24) Neilson, A. H.; Hynning, P.-A. *Handb. Environ. Chem.* **1998**, *3*, 223−269.

(25) Allamandola, L. J.; Tielens, A. G. G. M.; Barker, J. R. *Astrophys. J.* **1989**, *71*, 733−775.

(26) NRC. *Alternatives for Groundwater Cleanup*; National Research Council: Washington, DC, 1994.

(27) NRC. *Contaminated Sediments in Ports and Waterways, Cleanup Strategies and Technologies*; National Research Council: Washington, DC, 1997.

(28) NRC. *Innovations in Groundwater and Soil Cleanup*; National Research Council: Washington, DC, 1997.

(29) Arey, J. *Handb. Environ. Chem.* **1998**, *3*, 347−388.

(30) Handschuh, M.; Nettesheim, S.; Zenobi, R. *J. Phys. Chem. B* **1999**, *103*, 1719−1726.

(31) Li, Y.; McIver, R. T., Jr.; Hemminger, J. C. *J. Chem. Phys. 100*-, *93*, 4719−4723.

(32) Kinsel, G. R.; Lindner, J.; Grotemeyer, J.; Schlag, E. W. *J. Phys. Chem.* **1991**, *95*, 7824−7830.

(33) McGlothlin, D. R. Master's Thesis, Stanford University, Stanford, CA, 2003.

Physics Lasers, Mountain View, CA) is passed through the plume of desorbed molecules after a fixed delay period whose value is adjustable. This UV light selectively ionizes the aromatic molecules present in the plume through a (1+1) REMPI scheme. The resulting ions are analyzed in a modified Wiley−McLaren reflectron time-of-flight mass spectrometer that is equipped with microchannel plate detectors (Burle Electro-optics, Philadelphia, PA). The ion signals as a function of arrival time produced from 25 desorption shots are averaged together on a digital oscilloscope (Waverunner LT342, LeCroy, Chestnut Ridge, NY), and this average mass spectrum is then used for analysis. Peak areas are integrated using IGOR software (WaveMetrics, Lake Oswego, OR).

In this work, we investigated the effect of several μL^2MS operational parameters on the ratios of the peak areas of various PAHs. Both synthetic samples and "real-life" samples were analyzed to determine whether different substrates showed similar results. The parameters studied were as follows: (1) intensity of the desorption laser; (2) wavelength and spot size of desorption laser; (3) delay time between desorption and ionization; (4) intensity of the ionization laser; (5) alignment of the ionization laser.

Desorption laser intensity was controlled by a series of copper wire meshes similar to that described by Hanton.[34] Laser power was measured by a power meter (Scientech, Boulder, CO) prior to entry into the μL^2MS vacuum chamber. Desorption wavelength was controlled by the choice of the desorption laser as described above. The Er:YAG laser had a shorter wavelength and was also capable of producing higher power than the CO_2 laser.

The time delay between the desorption and the ionization steps is controlled by a pulse generator with adjustable delay (Stanford Research Systems, Sunnyvale, CA). The Nd:YAG ionization laser is triggered at its optimal rate (10 Hz) and serves as the master system clock. To permit single desorption shots, the trigger pulses for the desorption laser first pass through a home-built pulse repressor/pulse synchronizer box that allows the trigger to continue to the laser only when a switch is toggled by the user. Delay times ranging from 10 to 40 μs were used between the CO_2 laser trigger and the Nd:YAG trigger. The Er:YAG desorption laser requires more time after triggering to fire (an additional 560 μs), so delays of between 570 and 600 μs were used, corresponding to 10−40-μs delays between actual desorption and ionization, which is the same as for the CO_2 laser.

Ionization laser intensity was attenuated by a combination of a half-wave plate and a calcite polarizer. Power was again measured by a power meter prior to entry into the μL^2MS chamber. Shot-to-shot variation was ∼10%.

Alignment of the ionization laser is done by manually adjusting the position of the ionization laser as it passes through the ionization/extraction region of the instrument. The "optimal" alignment is found qualitatively. Gaseous toluene-d_8 (used as a mass calibrant) is introduced into the chamber, and the position of the Nd:YAG laser beam is adjusted horizontally and vertically until the maximum signal and minimum peak width is observed. Alignment has been observed in this laboratory to drift over the course of a day. To quantify the effects of ionization laser alignment in this work, the laser was adjusted vertically or

horizontally from this optimal position, and μL^2MS spectra were measured at these different alignments.

Sample Preparation. *PVC Membranes.* Synthetic samples of PAHs in poly(vinyl chloride) (PVC) membranes were prepared following the procedure outlined by Haefliger and Zenobi.[13] All chemicals were purchased from Sigma-Aldrich (Milwaukee, WI). In brief, 1 mL of a 60 mg/mL solution of high-weight PVC (average molecular weight 43 000) in tetrahydrofuran (THF) was combined with 1 mL of a PAH solution in THF. The PAH solution contained naphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene, phenanthrene, and pyrene (each 4×10^{-5} M) and chrysene and benzo[a]pyrene (each at 4×10^{-4} M concentration). These PAHs were chosen to cover a wide range of molecular masses (from 128 to 252 Da) and structures (from two rings to five rings) The PVC/PAH mixture was poured into a glass Petri dish, and the solvent was allowed to evaporate. After ∼1 h, the membrane was ready for measurements. Pieces ∼5 mm in diameter were cut and analyzed. It has been reported that volatile PAHs escape from such membranes over time,[14] causing the relative amounts of different PAHs to vary. Control experiments (not shown) were conducted to verify that the measured PAH ratios were stable over the time period required for analysis and variation of a given parameter.

Murchison Meteorite. Powdered samples of the Murchison meteorite were used as a representative "real-world" sample to compare with the synthetic PVC membrane samples. The Murchison meteorite has previously been shown to contain a variety of PAHs.[5,23] In this work, we analyzed the areas of the peaks corresponding to naphthalene, methylnaphthalene, and C_2-naphthalene (dimethylnaphthalene or ethylnaphthalene), phenanthrene, methylphenanthrene, and pyrene. It is not possible to determine the structure of the alkylated naphthalenes because μL^2MS is incapable of distinguishing between isomers at a fixed ionization wavelength. A small chunk (∼100 mg) of the Murchison meteorite (U.S. National Meteorite Collection, Smithsonian Institute) was crushed using an alumina mortar and pestle, and the powder was thoroughly mixed to form a homogeneous sample reservoir. Samples for analysis were prepared by causing a portion of the Murchison powder to adhere to a piece of double-sided tape mounted on a brass sample platter. Because previous studies have reported a change in analyte peak area ratios measured over time on soil samples,[11] control experiments (not shown) were performed to ensure that PAH ratios were stable for the number of spectra taken from a single sample in this study.

RESULTS AND DISCUSSION

The effects of each μL^2MS operating parameter are presented here as the change in the ratios of PAH peak areas determined under different conditions. The PVC membranes contained seven PAHs, whereas six PAHs were measured in the Murchison sample. In each case, the data presented show the PAH signal strengths normalized to naphthalene (the smallest PAH). To compare how PAHs of different masses, structures, and vapor pressures are affected, some data are also shown normalized to phenanthrene (a three-ring PAH) and, for the PVC membranes, to benzo[a]pyrene (the largest PAH measured, a five-ring compound).

Effects of Desorption Power, Desorption Wavelength, and Delay Time. Desorption laser intensity and wavelength influence

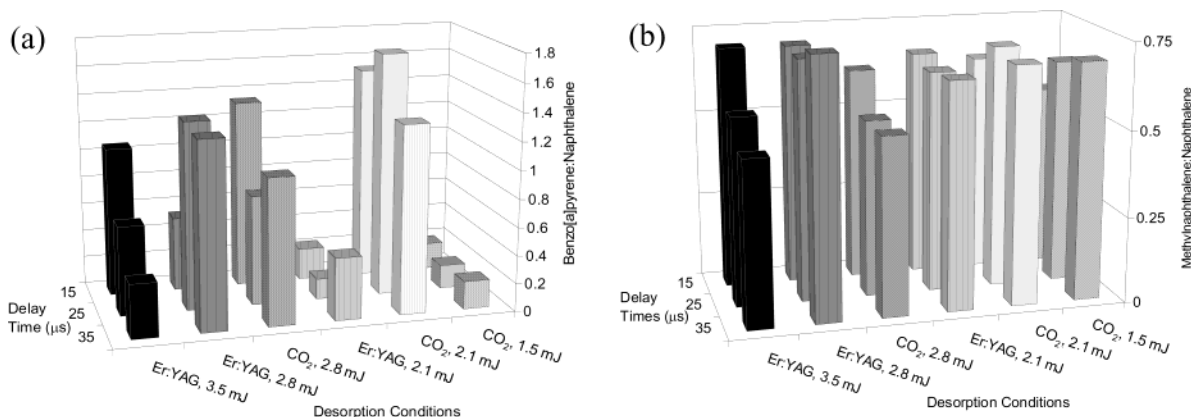(34) Hanton, S. D. *Rev. Sci. Instrum.* **1993**, *64*, 1456−1458.

Figure 1. Peak areas normalized to naphthalene for (a) benzo[*a*]pyrene and (b) 2-methylnaphthalene in PVC membranes under 18 combinations of desorption laser wavelength, desorption laser power, and desorption/ionization delay times.

both the efficiency of desorption and the kinetic energy profile of the desorbed neutrals. Changes in this profile affect the optimal delay time between desorption and ionization. Because of the interrelatedness of these three parameters, a complete data set examining the effect of each parameter was taken from each individual sample, and the resulting PAH ratios will be presented as a function of all three parameters.

PVC Membranes. PAH ratios were measured at six different desorption laser conditions: (1) CO_2 laser, 2.8 mJ/pulse; (2) CO_2, 1.9 mJ; (3) CO_2, 1.4 mJ; (4) Er:YAG, 3.8 mJ, (5) Er:YAG, 2.9 mJ; and (6) Er:YAG, 2.1 mJ. For each set of desorption conditions, delay times of 15, 25, and 35 μs between desorption and ionization were used. Three 25-shot averages were taken for each desorption/delay combination, leading to a total of 54 25-shot averages taken from the PVC sample. Control experiments (not shown) verified that the PAH ratios in the sample were stable for this number of shots. PAH ratios were calculated for each 25-shot average, providing a set of three resulting values for each desorption/delay combination. The average and standard deviation of these three values are used in the following plots.

Figure 1a shows the benzo[*a*]pyrene/naphthalene ratio as a function of the desorption and delay conditions measured. The ratio varies from a low value of 0.15 (Er:YAG, 2.1 mJ, 25 μs) to a high value of 1.7 (CO_2, 2.1 mJ, 25 μs), changing by more than a factor of 10. The measured ratios show striking differences for the two lasers at the same nominal pulse power. This result can perhaps be explained by examining the IR absorption spectrum of PVC at 2.94 (Er:YAG) and 10.6 μm (CO_2). The spectrum supplied by Sigma-Aldrich indicates that PVC is nearly transparent at 2.94 μm but absorbs strongly at 10.6 μm. Because the thermal desorption mechanism involves absorption of the incident laser radiation by the PVC substrate,[16] this difference in absorption leads to differing heating rates and, thus, different rates of PAH desorption. These changing rates of PAH desorption apparently affect naphthalene and benzo[*a*]pyrene to varying extents, changing the measured ratio between them.

Changing the delay time also has an effect on the ratio of benzo[*a*]pyrene to naphthalene, although it is not as pronounced as that caused by changing desorption conditions. In the most extreme case, decreasing the delay time from 35 to 15 μs when using the Er:YAG at 3.5 mJ/pulse nearly triples the ratio from 0.38 to 1.06. In other cases, decreasing the delay time decreases

the ratio. At the lowest desorption pulse powers, a change in delay time does not markedly affect the PAH ratio. No discernible way appears to allow prediction of the effect of delay time. Changing the delay time is equivalent to sampling the plume of desorbed neutrals at different locations. The measured change in the benzo-[*a*]pyrene/naphthalene ratio indicates that the composition of the desorbed plume is not constant, that different PAHs desorb at different rates, and that μL^2MS measurements reflect only the composition of the plume at a given time, rather than the actual composition of the sample.

In contrast to the variations observed in the benzo[*a*]pyrene/naphthalene ratio, Figure 1b presents the 2-methylnaphthalene/naphthalene ratio under the same desorption/delay conditions. It is immediately clear that this ratio is far less sensitive to changes in operating parameters than is the benzo[*a*]pyrene/naphthalene ratio. The 2-methylnaphthalene/naphthalene ratio varies between a low value of 0.48 (Er:YAG, 3.5 mJ, 35 μs) and a high value of 0.75 (Er:YAG, 2.8 mJ, 35 μs), a factor of 1.6 change. In this case, differences in laser wavelength do not change the ratio noticeably (compare Er:YAG and CO_2 at 2.1 mJ/pulse). Laser power appears to be a factor, with the highest powers of Er:YAG (3.5 mJ) and CO_2 (2.8 mJ) producing lower ratios than those observed at lower powers. Delay times appear significant only at these highest powers.

An examination of Figure 1a and b suggests that changing the desorption and delay conditions affects the measured amounts of PAHs and that different PAHs respond to these changes to differing degrees. Benzo[*a*]pyrene and naphthalene, two PAHs that differ vastly in size and mass, respond quite differently, leading to large fluctuations in the ratio of these two compounds. 2-Methylnaphthalene and naphthalene are more similar in size and mass, and the ratio between these compounds is more stable. By defining a "variation factor" as the maximum value of the ratio divided by the minimum value measured under the different desorption/delay conditions, it is possible to show the correlations between PAHs. Figure 2 presents the variation factors for the ratio of each PAH compared to naphthalene, phenanthrene, or benzo-[*a*]pyrene. It is clear that similar PAHs show less variability; for example, the 2-methylnaphthalene/naphthalene ratio, the pyrene/phenanthrene ratio, and the chrysene/benzo[*a*]pyrene ratio all have variabilities of 2 or less. On the other hand, ratios of dissimilar PAHs such as benzo[*a*]pyrene/naphthalene, chrysene/
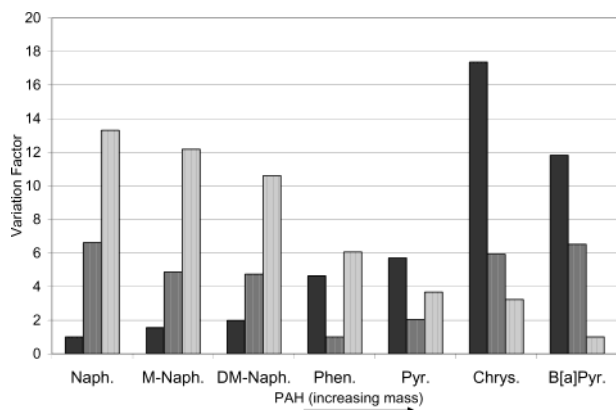
Figure 2. Variation factor for the ratios of the given PAHs (naphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene, phenanthrene, pyrene, chrysene, and benzo[*a*]pyrene) to naphthalene (■), phenanthrene (dark gray column), and benzo[*a*]pyrene (light gray column) in PVC membranes over the range of desorption and delay conditions shown in Figure 1. Variation factor is defined as the maximum value of the ratio divided by its minimum value.

naphthalene, or methylnaphthalene/benzo[*a*]pyrene vary by more than a factor of 10. One cause for this behavior could be differences in vapor pressure, which varies inversely with size. The vapor pressures of benzo[*a*]pyrene and naphthalene differ by 10 orders of magnitude,[35,36] and it is possible that vapor pressure is related to volatility and ease of desorption.

Although it is relatively easy as a practical matter to control the desorption and delay conditions, the trends revealed by changing these parameters for the PVC samples illustrate three findings that have significant bearing on the understanding and applications of quantitative μL^2MS. First, the measured ratio between two PAHs may not accurately represent the actual ratio of the compounds in the sample. In the sample presented here, the amount of benzo[*a*]pyrene in the PVC membrane was 10 times greater than that of naphthalene, but the measured ratios ranged from 0.15 to 1.7. Second, the plume of desorbed molecules does not have a homogeneous composition, and the portion of the plume sampled by μL^2MS is affected by operational parameters. Finally, dissimilar PAHs respond differently to changing conditions, meaning that the degree of similarity between an analyte and the internal standard chosen for quantification will affect the reliability and variability of the measured ratio and, hence, the quantification.

Murchison Meteorite Powder. The Murchison powder was analyzed under five desorption conditions: (1) CO_2 laser, 2.8 mJ/ pulse; (2) CO_2, 2.1 mJ; (3) CO_2, 1.5 mJ; (4) Er:YAG, 2.8 mJ; (5) Er:YAG, 2.1 mJ. Seven delay times from 10 to 40 μs in 5-μs steps were measured, yielding a total of 35 data points. Again, three 25-shot averages were taken for each point, and control experiments verified that the sample did not degrade over the time needed for this analysis.

Figure 3 displays the ratios for pyrene/naphthalene and methylnaphthalene/naphthalene. As with the PVC samples, a large variation occurs in the measured PAH ratios as desorption and delay conditions change. The variability of the ratios is much

greater for Murchison than for the synthetic PVC samples, with the pyrene/naphthalene ratio changing from a low value of 0.18 (CO_2, 2.8 mJ, 35 μs) to a high of 4.5 (CO_2, 1.5 mJ, 10 μs). This result implies a factor of 24 change, compared to the factor of 10 variability seen for the benzo[*a*]pyrene/naphthalene ratio in the PVC membrane. This increased dependence of the ratio on desorption and delay conditions may reflect the differences in desorption heating profiles between the inhomogeneous mineral matrix of the Murchison powder and the more homogeneous PVC matrix.

Desorption wavelength is again linked to differences in PAH ratios, with the Er:YAG tending to show relatively more pyrene than that of the CO_2 laser at the same pulse power. The CO_2 laser also creates desorption plumes that are more sensitive to delay times than the Er:YAG; the pyrene/naphthalene ratio varies greatly from 10 to 40 μs for the CO_2 laser at a given pulse power, whereas it remains relatively constant for the Er:YAG laser. In fact, for the data taken with the CO_2 laser, a clear trend is observed in which the relative amount of pyrene to naphthalene increases as the desorption power is lowered and the delay time is shortened. Intuitively, one might expect the smaller, more volatile naphthalene to be desorbed more easily and quickly, causing a trend that is the opposite of that observed. An examination of the raw data (not shown) shows that whereas the amount of pyrene in the plume peaks in the first 15 μs, the amount of naphthalene remains constant, even slightly increasing with longer delay times. One possible explanation is that heating of the Murchison powder by the CO_2 laser permits the volatile naphthalene within the sample to be transported to the surface and vaporized, creating a nearly constant supply of naphthalene in the plume. The larger, less volatile PAHs cannot move through the matrix as easily, so the plume contains only the surface concentration of these compounds. This effect is not seen with the PVC samples, nor with the Er:YAG, suggesting that the combination of desorption wavelength and substrate composition can change the depth of sampling observed for different PAHs in the desorption plume.

The variability in PAH signal strength also seems to correlate with PAH size, mass, and vapor pressure, as was seen in the PVC samples. The relative amounts of methylnaphthalene and naphthalene vary by only a factor of 2.4 (from 0.17 for the CO_2 laser, 2.8 mJ, 20 μs, to 0.41 for CO_2, 1.5 mJ, 10 μs), compared to the factor of 24 seen for pyrene and naphthalene. Figure 4 shows the variation factors for the measured PAH ratios relative to naphthalene and phenanthrene. Again, the ratios of similar PAHs such as naphthalene and its alkylated derivatives or phenanthrene and methylphenanthrene show relatively little variation, whereas the ratio of dissimilar PAHs such as pyrene and naphthalene vary significantly with changing conditions.

The Murchison data provide additional cautionary information for the analysis of quantitative μL^2MS. It appears that "real-life," inhomogeneous sample surfaces are much more sensitive to changes in μL^2MS operational parameters. Although these samples have been among those heavily studied by μL^2MS in the past, this may impose some limitations on the applicability of quantitative μL^2MS analysis to these samples. The differing responses exhibited by dissimilar PAHs also raise concerns about the possibility of using a single internal standard to quantify PAHs with μL^2MS.

(35) Agency for Toxic Substances and Disease Registry; U.S. Department of Health and Human Services, Public Health Service: Atlanta, GA, 1995.

(36) Shiua, W.-Y.; Mab, K.-C. *J. Phys. Chem. Ref. Data* **2000**, *29*, 41−130.
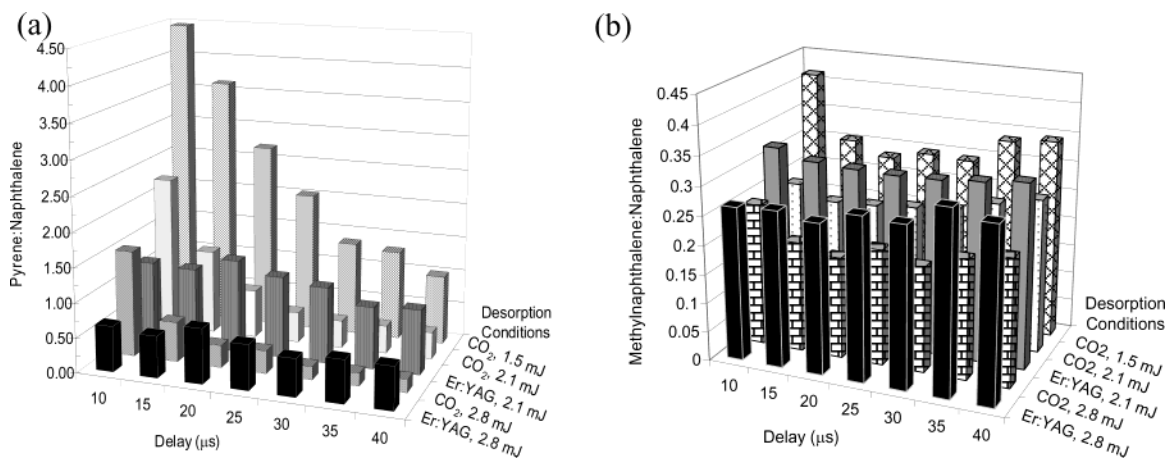
Figure 3. Peak areas normalized to naphthalene for (a) pyrene and (b) methylnaphthalene in Murchison meteorite powder under 35 combinations of desorption laser wavelength, desorption laser power, and desorption/ionization delay times
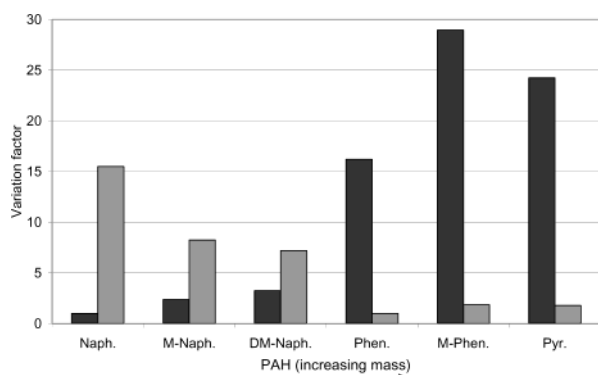

Figure 4. Variation factor for the ratios of the given PAHs (naphthalene, methylnaphthalene, C_2-naphthalene, phenanthrene, methylphenanthrene, pyrene) to naphthalene (■) and phenanthrene (gray column) in Murchison meteorite powder over the range of desorption and delay conditions shown in Figure 3. Variation factor is defined as the maximum value of the ratio divided by its minimum value.

Ionization Laser Power. The effect of ionization laser power on the PAH ratios was investigated by analyzing the PVC membranes and the Murchison powder with the UV laser at 3, 4, and 5 mJ/pulse. Figure 5a presents the ratios of the measured PAHs compared to naphthalene in the PVC sample, and Figure 5b shows the ratios for the Murchison sample. In both cases, significant changes in the ratios are observed upon variation of the UV laser power.

As laser power increases from 3 to 5 mJ, the ratios drop by as much as a factor of 4 in the PVC (benzo[*a*]pyrene/naphthalene or chrysene/naphthalene) and by a factor of 2 in the Murchison sample (pyrene/naphthalene). As with the variability caused by changes in desorption and delay conditions, the amount of variation in PAH ratios is linked to the similarity of the PAHs being compared. The alkylnaphthalene/naphthalene ratios vary only slightly, while the ratios between the largest PAHs and naphthalene show significant changes.

Variability caused by fluctuations in laser ionization power is highly relevant to understanding the limitations and abilities of quantitative μL^2MS, because this parameter is relatively difficult to control. Shot-to-shot fluctuations in ionization laser power as high as 20−50% have been reported.[12] Various quantitative μL^2-

MS studies have compensated for ionization laser fluctuations by normalizing the PAH signal to the laser power of each shot.[7,13] This method is acceptable for quantification of individual PAHs that show linear responses to ionization power or for qualitative work, but appears to be inadequate when comparing the ratios of two PAHs that may have different linear responses caused by differences in photoionization efficiency. This finding represents a significant caveat for design of quantitative μL^2MS methods using internal standards.

Ionization Laser Alignment. The effect of changing the alignment of the ionization laser beam as it passes through the extraction region of the μL^2MS was also investigated. A change in laser alignment corresponds to sampling different areas of the plume of desorbed molecules. The optimal alignment is qualitatively chosen as that which gives the maximum signal for gaseous toluene-d_8, a mass calibrant used in this μL^2MS system. Alternative alignments were created by altering the position of the beam vertically or horizontally in the extraction region.

Figure 6 presents the PAH ratios, computed relative to naphthalene, for several alignments used in analysis of the Murchison sample. Figure 6a shows the change in ratios seen upon vertical translation of the ionization beam over a distance of 4 mm up and down from the optimal position. Figure 6b displays the variability caused by horizontal translation by 2 mm in each direction from the optimal alignment. Significant variations are seen, with the measured PAH ratios changing by up to a factor of 2.6 in the vertical direction and up to 10.7 in the horizontal direction. The degree of variability once again shows a correlation with PAH sizes, masses, and vapor pressures. Ratios of similar PAHs (methylnaphthalene/naphthalene) show smaller changes than those of dissimilar PAHs (pyrene/naphthalene). Similar variations in PAH ratios caused by UV alignment are seen for the PVC membrane samples (not shown).

The data presented in Figure 6 indicate that the plume of molecules liberated in the desorption step is not homogeneous. This finding correlates well with the data collected by varying the delay times between the desorption and ionization steps. The plume shows definite compositional differences along both the horizontal and vertical axes. These differences could be caused by multiple factors, primarily those that influence how easily molecules are desorbed from the sample and how fast they travel
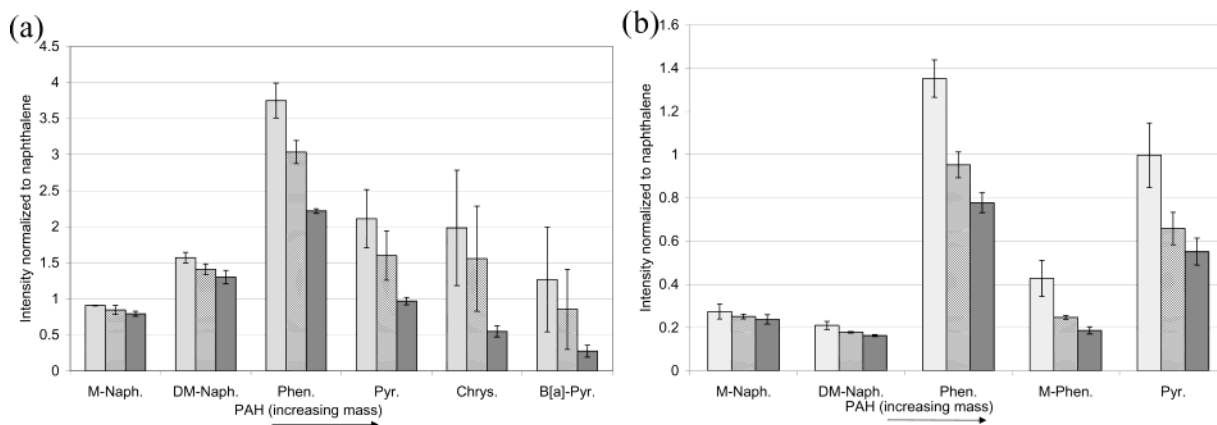
Figure 5. Ratios of the given PAHs to naphthalene in (a) PVC membranes and (b) Murchison meteorite powder at ionization laser powers of 3 (light gray colum), 4 (medium gray column), and 5 mJ (dark gray column).
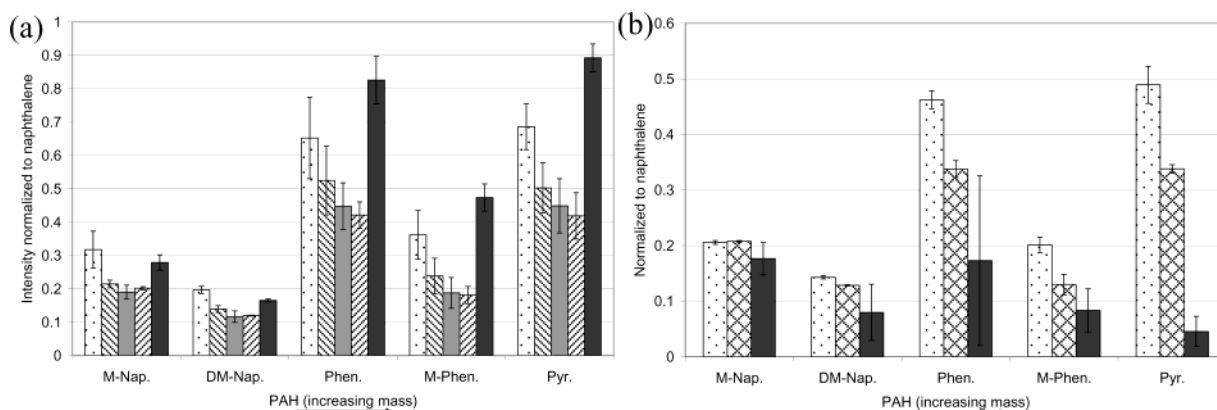

Figure 6. Ratios of the given PAHs to naphthalene in the Murchison meteorite powder for various alignments of the ionization laser. (a) shows vertical alignments of 4 mm below optimal position (dotted column), 2 mm below optimal (left to right hatch column), optimal alignment (dark gray column), 2 mm above optimal (right to left hatch column), and 4 mm above optimal (■). (b) represents horizontal alignments of 2 mm toward the reflectron from optimal alignment (dotted column), optimal alignment (cross-hatch column), and 2 mm away from the reflectron from optimal (■).

once they have been desorbed. The topmost portion of the plume, sampled by the highest vertical alignment, would consist of molecules that either were among the first released in the desorption process (those having the most time to travel vertically) or those released with higher kinetic energy (those with enough speed to travel quickly to the top of the plume). Similarly, the lowest vertical alignment would sample either the molecules that are desorbed later or those with lower kinetic energy. Differences in measured PAH ratios along the horizontal direction could be explained by these same variations in desorption time and kinetic energy; they could also, however, be indicative of changes in ion extraction efficiency. Moving the ionization beam horizontally changes the position of ion formation in the extraction region of the mass spectrometer, potentially changing the relative efficiency with which ions of different masses are accelerated through the flight chamber and reflectron.

The UV laser alignment is an operating parameter that is not easily controlled. In our experience, alignment drifts over the course of a day as temperature changes and other variations in the environment occur. The dependence of PAH ratios on alignment may, therefore, require increased vigilance in maintaining a constant and reproducible laser alignment throughout the entirety of a quantitative μL²MS analysis. The amount of alignment variation shown in Figure 6 (up to 4 mm) is more than is likely to

occur in this time, but the trends shown by these data would apply on a smaller scale for smaller translations of the UV beam. Once again, the differing responses of PAHs of different sizes and structures to alignment fluctuations raise concerns about the use of a single internal standard as a means of quantification.

CONCLUSIONS

The sensitivity, selectivity, and relative ease of sample preparation involved in μL²MS analysis has led to much recent interest in developing quantitative μL²MS techniques. In this work, we have presented some characteristics of μL²MS responses to various operational parameters that illustrate some of the limitations and challenges facing the development of quantitative μL²-MS. Many of the recently developed methods rely on comparison of the signal strength of an analyte to that of an internal standard; our work has shown that this ratio can be highly variable, depending on such factors as desorption laser power and wavelength, delay time between desorption and ionization, and ionization laser power and alignment.

The variability in signal ratios for PAHs exists in both synthetic samples similar to those used in previous quantitative μL²MS studies and the more heterogeneous solid samples that have been extensively analyzed by nonquantitative μL²MS. The extent of ratio variation differs between these samples, indicating that differences

in substrate properties are another important factor to consider when analyzing quantitative μL^{2}MS data.

Although this work indicates that individual PAHs respond to different extents to parameter changes, thus causing changes in their ratios, we have also shown that PAHs that are similar in mass, structure, and vapor pressure display similar responses. This finding is a key point to consider in developing quantitative μL^2-MS methods, as it suggests that internal standards should be closely related to the analytes of interest for reliable measurement of signal ratios. Multiple internal standards may be needed to quantify samples that contain a large number of different PAHs with an acceptable level of accuracy. Our data also imply that measurements of the degree of alkylation of PAHs should be possible, as the ratios of alkylated PAHs to their nonalkylated parent structures remain stable within a factor of 2 with parameter changes.

The factors shown here to influence PAH ratios can be controlled to a large extent. Some fluctuations, however, are more difficult to eliminate. In particular, stabilization of shot-to-shot variations in ionization laser power is not an easy task. These variations, as well as smaller instabilities in ionization laser alignment and desorption laser power, will contribute to inherent fluctuations in the μL^{2}MS instrument, leading to variability in PAH ratios and difficulties in quantification. This behavior may explain why previously reported results for quantitative μL^{2}MS measurements report uncertainties ranging from 7 to 35%, with some duplicate sample measurements differing by more than 200%.[12,14] It appears that these inherent fluctuations may impose fundamental limits on the quantitative abilities of μL^{2}MS.

μL^{2}MS has shown itself to be a useful technique in the qualitative analysis of a variety of environmental and extraterrestrial samples. The extension of μL^{2}MS to quantitative measurements would be a valuable addition. By revealing some of the factors affecting μL^{2}MS responses and the challenges they pose, we hope that this work will aid in understanding the limitations of μL^{2}MS for quantitative analysis. Conversely, this understanding forms the basis for improved designs to make μL^{2}MS measurements more quantitative.