FUNDAMENTAL STUDIES WITH A MONODISPERSE AEROSOL-BASED
LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY INTERFACE
(MAGIC-LC/MS)

Progress Report

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Studies with Thermal Vaporization Properties of Particle Beam Interface

One of the most important aspects of particle beam LC/MS is the interaction of the solvent free particle with the heated ion source chamber. Our studies have demonstrated that the particles reaching the ion source region of the mass spectrometer are typically in the size range of 20-100 nm diameter. Particles of this size vaporize rapidly at the temperature of the ion source, after collision with a heated wall surface. However, the precise nature of this interaction, the time period for which the particle adheres to the wall, the rate of energy transfer from the wall to the particle and the subsequent transfer of the molecular species into the vapor phase are not well characterized. Consequently, we have carried out a series of studies with a various test compounds in order to determine the influence of temperature on the vaporization and decomposition properties of these molecules.

We have selected both thermally stable and thermally labile molecules in order to establish a framework for estimating the competitive rates at which molecules are thermally desorbed from a heated surface and at which they absorb internal energy. The absorption of thermal energy solely in the molecule/surface bonds will result in production of intact vapor phase species, whereas absorption of energy into the internal (vibrational) states of the molecule will result in substantial decomposition of the molecule, and probable loss of the molecular ion. The test compounds used were a series of polynuclear aromatic (PAH) compounds covering a wide range of molecular weights from two-ring naphthalene (128 amu) to twelve-ring perylene (531 amu). The vaporization properties of these compounds show little evidence of thermal degradation under any conditions, as they are extremely thermally stable, and so make an excellent reference point for comparison with less thermally stable species. The less thermally stable species examined were aldicarb, a highly thermally labile pesticide and cholesterol, which readily loses water when subjected to high temperatures. The appearance of the mass spectra of these two molecules under conditions of thermal decomposition are for aldicarb the decrease in the magnitude of the molecular ion peak, and for cholesterol a reduction in the ratio of the intact molecular ion (386 amu) to the intensity of the molecule minus a water molecule (368 amu). The ratio for a near ideal situation would have a 386/368 ratio of approximately 4:1.

Our studies have show some very interesting trends to date. While we have not as yet explored the situation at very high temperatures, and have run only at temperatures of < 325 °C, nevertheless, the extent of thermal decomposition has been shown to have a direct relationship to the mass loading of material on the heated ion source surface. Mass loading is controlled by varying the mass of analyte species injected into the LC injection loop. As the mean diameter of the generated aerosol is known, this allows the calculation of the mean diameter of the desolvated particle reaching the heated ion source of the mass spectrometer. It has been shown by these studies that the production of intact molecular ions from thermally labile species bears a direct relationship to the particle size reaching the heated surface, which in turn is directly related to the effective film thickness on the surface. The thinner the film thickness, the faster the vaporization process which occurs, and so the more rapidly the molecule/surface bonds are broken and the

less prevalent thermal decomposition of the molecular species becomes. This has been demonstrated to lead to a remarkable reduction in, for example, the ratio of 386/368 ions for cholesterol. At high mass loadings (1 µg injected) and high temperatures (325 °C) the 386/368 ratio may be as low as 2.0, whereas at lower mass loadings (100 ng injected) the 386/368 ratio is close to the "ideal" value of 4.0.

Further studies are being pursued in which a new, externally heated probe, which can be raised to temperatures as high as 500 °C will be used. It is hoped that by increasing the rate of desorption from the heated surface to even high values that the time of residence of thermally labile species on the high temperature surface will be further reduced, and will allow the production of relatively intact vapor phase molecules from higher molecular weight species than had been thought possible previously using EI or CI mass spectral generation. Additional studies in this area will also examine the very interesting observation that the vaporization of high molecular weight species, such as Gramicidin S (m.w. 1152 amu) are significantly enhanced in the CI mode compared to the EI mode of ionization. The primary difference between these two modes of ionization is the pressure in the ion source. For EI spectral generation it is typically 2×10^{-5} torr, whereas for CI mode it will be in the high millitorr to low torr range. We believe that the enhancement of signal in CI mode for highly involatile species is primarily due to this pressure increase, and we intend to investigate whether control of pressure may be a major factor in which could be used in enhancing the vaporization of involatile, high molecular weight species. This is an aspect of particle beam LC/MS which is receiving particular attention, as attempts are being made to extend its range of applicability to ionic compounds of environmental and biological interest.

Fast Atom Bombardment Particle Beam LC/MS

Combining particle beam mass spectrometry with FAB mass spectrometry has proved to be a promising approach for the generation of spectra from non-volatile compounds.¹ The great potential for on-line HPLC FAB mass spectrometry system has created the need for better understanding of the fundamental processes which occur in the particle beam LC/FAB MS system. The role of the matrix has been, and will continue to be, a major point of investigation. One specific area of concern is matrix loading. Interest in the influence of matrix loading on FAB signals achieved stimulus when Caprioli reported a significant improvement in S/N with continuous flow FAB using low percentages of matrix, e.g.(5%-20%).² Since that time, Heine has studied matrix loading in static FAB, again finding improvements in S/N with low percentages of matrix.³ During the last year, our research has addressed matrix loading effects for a range of surface active and non-surface active compounds in three different matrices: glycerol, 3-nitrobenzyl alcohol, and thioglycerol. Additionally, the time dependence of FAB spectra generation in the particle beam system has been examined and contrasted with ion generation in normal probe FAB work.

PB LC/FAB MS provides a valuable approach for the analysis of non-volatile compounds. Compounds are ionized by the FAB beam, and spectra result which are similar to spectra produced using classic techniques. Samples are introduced into the source of the mass spectrometer in a relatively straightforward manner. Also, the ability to control the amount of matrix added not only reduces the amount of background due to the matrix, but also decreases contamination of the source. When the presence of matrix with the sample is detrimental to the chromatography, post column addition of matrix may be

performed with little loss of chromatographic integrity. Addition of matrix through the target produces higher sensitivity and no corruption of the chromatography; however, the frequency of source maintenance will increase due to contamination from continual bombardment of the matrix.

Under flow injection conditions, the system displays a high degree of stability over several hours. The degree of stability is, however, dependent on the mode of matrix addition, the type of matrix used and the amount and type of sample used. When matrix is added to the sample, stability is affected by three things, type of matrix, amount of matrix, and number of injections. The more involatile matrices like glycerol tend to collect on the skimmers in the momentum separator, reducing transport efficiency. The end result is that signal decreases as the number of injections increases. More volatile matrices like thioglycerol and 3-nitrobenzyl alcohol are less likely to collect on the skimmers and this gives better stability for a given number of injections; however, some form of blockage is eventually likely to occur, ultimately leading to a reduction in analyte transport. In this situation the matrix loading will determine how many injections are possible before stability suffers. Experimentally, it is observed that after several injections, the target becomes stained. This is a result of matrix interaction with the FAB beam. It is not clear whether this process affects the signal stability; however, background was observed to increase slightly during this period.

PB LC/FAB MS is useful for a wide range of compounds, although there is considerable variation in response between them. Response is dependent on the choice of compound, matrix, and ionizing gas, as well as the performance of the interface, and the condition of the mass spectrometer. Response varies greatly with compound type. Aerosurf (a quaternary ammonium salt) deposited on the matrix gave a detection limit of 50 ng, while several micrograms of the azo dyes were necessary to produce useful spectra when deposited onto the matrix. This behavior may be related to a number of factors, including surface activity, gas-phase basicity, and insolubility in the matrix. Response is also dependent on the matrix used. This was evident with erythromycin, which showed relatively good response using "magic bullet" as matrix, poor response with NBA, and no response with glycerol. The performance of the interface will also affect response through changes in transport efficiency. The condition of the mass spectrometer may also exert a major effect on response. Specifically, the tune of the mass spectrometer is critical, and varies considerably from matrix to matrix, especially when matrix is placed on the target. For example, thioglycerol requires a completely different set of tune values compared to glycerol. This is most likely explained by the difference in vapor pressure of the two compounds.

Results from studies involving matrix loading showed that response is dependent upon the ratio of matrix to analyte. An order of magnitude increase in signal was observed when the optimum amount of matrix was used. Quantitative response was found to be dependent upon available surface area. It was shown that a 50% reduction in the amount of matrix resulted in a substantial decrease in linearity. A linear plot of concentration vs. peak area for the matrices studied showed a concentration effect similar to that seen with Beer's law. At higher concentrations the surface area was insufficient to accommodate additional analyte, and so the curve bent back towards the concentration axis.

It was also observed that better sensitivity was obtained when matrix was placed on the target, rather than combined with the sample. There are two explanations which can account for the observed results. When matrix is mixed with the sample, the viscosity of the sample is changed. Changes in the viscosity will produce changes in the drop size distribution upon sample nebulization. If the droplet size is increased significantly, then analyte transport will be decreased, causing a reduction in sensitivity. A second possibility is that the particle beam from the interface is deposited on the surface of the matrix only, with no penetration of the surface. On the surface, analyte is independent of diffusion processes and is more readily ionized by the FAB beam. Because Aerosurf is a surfactant it will inherently tend to reside on the matrix surface, and so it is difficult to determine whether or not it penetrates the surface of the matrix. Erythromycin, which has much less surface activity, was therefore used to compare its properties with those of Aerosurf. When injections were made onto the NBA matrix, initially very small signals were seen. After 5 minutes of fast atom bombardment, the signal increased to a maximum and then rapidly decreased. Apparently the erythromycin was able to diffuse to the surface only when a substantial amount of matrix had evaporated. These findings indicate that the particle beam penetrates the surface of the matrix; however, earlier studies using "magic bullet" produced well defined peaks immediately after injection. Further study is necessary to determine the interaction which is occurring between the particle beam and the surface of the matrix. This type of phenomenon must be explained in order to produce the performance and predictability necessary for on line chromatographic analysis.

Future FAB LC/MS Research

At the present state of development of the FAB work, a number of general conclusions may be drawn: 1) PB LC/FAB MS is a novel and versatile approach for the production of ions from semi-volatile and non-volatile compounds. 2) Spectra produced with LC/FAB MS are similar to, if not always identical to spectra obtained using classic or static FAB MS. 3) There are two viable ways to operate PB LC/FAB MS (a) with matrix added to the sample or mobile phase, and (b) with matrix placed directly on the target. 4) The system shows acceptable stability over the time periods necessary for chromatographic runs, although the lifetime of volatile matrices placed directly on the target may not be adequate. 5) PB LC/FAB MS is applicable to a wide range of compounds. However, response varies significantly, depending on the nature of both the compound and the matrix. 6) Chromatographic separations are possible, however, greater response per mass of analyte injected is necessary to avoid poor chromatographic peaks due to overloading the column. 7) Response is dependent on the ratio of analyte to matrix. 8) Quantitative response is dependent on the available matrix surface area and the concentration of analyte on the surface. 9) Mechanistically, it appears that the particle beam penetrates the surface of the matrix material collected on the probe. Analyte must therefore, diffuse back to the surface before it can be ionized by interaction with the atom beam.

A second interesting observation related to ionization involves an observed time lag between the appearance of the analyte ion signal and the matrix ion signal. With the mass spectrometer set to monitor both a matrix ion and an analyte ion in rapid sequence, samples containing matrix and analyte were injected and analyzed. Upon integration it was noticed that the initial appearance of the analyte signal always preceded the matrix signal. Sometimes the time lead was much as 1 second. This phenomenon merits further study for a wide range of analyte and matrix species. PB LC/FAB MS itself provides a unique way to study FAB mechanisms. While the ultimate goal of this research project is to produce a system which is

capable of on-line chromatographic analysis, a better understanding of the nature of the FAB process is central to successful development. To this end, rigorous experiments need to be carried out to determine the precise role of the matrix in the ionization process. Ideally, a number of species with widely different surface activities and gas-phase basicities should be studied. Novel approaches to the delivery of matrix to the target should also have a high priority. A probe similar to a continuous flow FAB probe has been built, which allows matrix to be added to the target without interrupting the experiment. A heater controller has also been built, so that the temperature of the probe may be controlled, thus controlling the evaporation rate of the matrix. A number of different matrices should also be explored, including the use of novel species such as liquid metals, (e.g. gallium). The enhancement of sensitivity should also have high priority. As more is understood about interactions of matrix and analyte, better sensitivity may result. Additionally, significant improvements may be expected from improvements in source design and in development of ion optics more suitable for particle beam/FAB of high molecular weight species.

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PUBLICATIONS UNDER PRESENT GRANT

Reviewed Journals (Citing DOE Support)

"Reversed phase and gradients separations capabilities of the particle beam LC/MS interface," D.D. Perkins, W.E. Harris and R.F. Browner, *Anal. Chem.*, in press 1990.

"Transport Properties of the Particle Beam LC/MS Interface," J.D. Kirk, K. Edman and R.F. Browner, *J. Amer. Soc. Mass Spectrom.*, submitted for publication 1990.

"Vaporization Processes for Involatile and Thermally Labile Species in Particle Beam Liquid Chromatography/mass Spectrometry," W.E. Harris, J. Small and R.F. Browner, *Anal. Chem.*, submitted for publication 1990.

Conference Presentations

"The Next Logical Step: LC/MS in Environmental Analysis," Richard F. Browner, 6th Waldbronner Themen, Enklosterle, West Germany, 1990 (**invited talk**).

"Particle Beam LC/MS for Environmental Monitoring," Environmental Teleconference, San Jose, CA, 1990 (**invited talk**).

"Particle Beam LC/MS Interfacing of Environmentally Important Compounds," W. Eric Harris and Richard F. Browner, American Society of Mass Spectroscopists Meeting, Tucson, AZ 1990.

"Fast Atom Bombardment Studies using Particle Beam LC/MS," J. Daniel Kirk and Richard F. Browner, American Society of Mass Spectroscopists Meeting, Tucson, AZ 1990.

"Supercritical Fluid Particle Beam Mass Spectrometry Interfacing for Biologically Important Compounds," American Society of Mass Spectroscopists Meeting, Tucson, AZ 1990.

"LC/MS Interfacing: a Marriage of Particle Dynamics and Particle Ionization"

Richard F. Browner, J. Daniel Kirk, W. Eric Harris and Katherine Edman, Eastern Analytical Symposium, Somerset, N.J. 1990 (**invited talk**).

"An Overview of Particle Beam LC/MS," Richard F. Browner, J. Daniel Kirk and W. Eric Harris, LC/MS Symposium, Montreux, Switzerland 1990 (**invited talk**).

"Supercritical Fluid Chromatography Interfacing with a Particle Beam Interface (SFC-PB-LC/MS): Application to Biochemically Important Compounds," Richard F. Browner, Diana Burdette and Gunther Holzer LC/MS Symposium, Montreux, Switzerland 1990 (**invited talk**).

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