

CHAPTER I

INTRODUCTION

1.1 Motivation

Since its inception in the mid-1980's, matrix assisted laser desorption/ionization (MALDI) has revolutionized analytical and biological chemistry by making possible the detection of large, intact biological molecules in the gas phase. This technique also motivated improvements in mass spectrometry, resulting in significant improvements in resolution and mass accuracy for biological analysis. These accomplishments were recognized with the partial award of the 2002 Nobel Prize in Chemistry.

While the contributions of MALDI to the study of biological molecules clearly represent the technique's most significant advances, MALDI is a generic analytical technique that has also been applied to a wide range of materials and disciplines. Improvements of the technique with regard to applications, equipment and new classes of compounds, continue to be developed. However, the analytical successes of MALDI have proceeded at a faster rate than the understanding of the fundamental processes, and there is every reason to expect that improved understanding of these processes, in addition to satisfying scientific curiosity, will motivate further advances. The fact that MALDI is a general process, accommodating a wide range of materials and experimental conditions, while making it particularly useful, complicates efforts to define the underlying mechanisms. In fact, it is generally acknowledged that a standard model of MALDI is not likely to exist.

Historically, laser mass spectrometry of biological molecules was limited those having molecular weights less than $\sim 10,000$ amu. Larger molecules could not be desorbed with laser energies beneath values that induced fragmentation. The key breakthrough realized in MALDI was the use of a matrix material to absorb laser energy and lead to the dissolution of the sample and liberation of the larger biomolecules into the gas-phase. The matrix was originally chosen to match an available laser wavelength that was also not strongly absorbed by the biomolecule, limiting the coupling of energy into the molecule that might result in undesirable photochemistry and fragmentation. The matrix also serves to assist in the ion formation of the analyte through

poorly understood mechanisms. Gas-phase ions are then typically analyzed by conventional mass spectrometric techniques.

It was recognized early in the development of MALDI that infrared lasers could perform effectively as light sources, and generally, IR-MALDI can be used in all respects that UV-MALDI can, though not always at the same level of performance. IR-MALDI has been shown to be advantageous over UV-MALDI, resulting in less fragmentation for some especially labile compounds [1-3], and for those compounds sensitive to photochemical modifications [4]. Additionally, IR-MALDI is attractive since many desirable matrix materials, especially those representing the environment of biological molecules, possess vibrational bands in the mid-infrared. However, due to present limitations IR-MALDI has remained a tool primarily for those involved in mechanistic studies, while most analytical and applications are accomplished with less expensive and more common ultraviolet lasers. The potential advantages of IR-MALDI provide motivation to better understand the critical parameters leading to a successful IR-MALDI experiment and to extend the technique to new model systems. The fact that IR-MALDI begins with significantly different initial excitation, and often physical, states provides a contrasting framework to UV-MALDI from which to consider the ablation and ion formation events.

1.2 Significance and original results of the present work

It is the objective of this body of work to better understand and explain the fundamental mechanisms responsible for successful IR-MALDI, and particularly to exploit the advantages provided by a tunable infrared laser source to provide another perspective from which to determine the applicability of current models of MALDI to processes initiated with infrared sources. The following is a summary of the principal results:

1. The most comprehensive study on the effect of irradiation parameters on mass spectra using the widest range of photon energies and pulse durations to date tested the importance of possible avenues of primary ion formation. The general observation is that in all cases, despite the wide range of initial conditions, similar spectra were observed, casting doubt on the plausibility of several putative models of ion formation.

- A range of ultraviolet photon energies tested the likelihood of multiphoton excitation of 2,5-dihydroxybenzoic matrix ions. The photon energies used accessed different excited states, and thus ion formation via two- or three-photon processes should show a strong dependence on fluence and/or photon energy; however, such a dependence was not evident in either the matrix or analyte spectra.
 - Laser pulses with similar fluences but temporal durations of femtosecond to nanosecond pulses were employed to test the influence of multiphoton excitation and excited state ionization. Despite the vast differences in photon fluxes employed, no increase in ion signal was observed for the high irradiance pulses.
 - The range of laser pulse widths also effectively tests the possibility that laser plume interactions contribute significantly to the observed ion yield. Given the known values of plume expansion, only the tail end of nanosecond, but not femtosecond, pulses are able to intercept the expanding plume. However, spectra taken with both pulse durations do not result in observable differences.
 - Vibrational and electronic excitation of the same model systems yielded spectra with qualitatively similar response in the analyte region and only minor differences in the low mass (matrix) region of the spectra.
 - As a whole, these results suggest that the observed ion species do not directly result from primary excitation events, but most likely are determined by other processes, most likely in the expanding plume after it has reached some degree of thermodynamic equilibrium.
2. Infrared excitation of organic molecules in sodium nitrate crystallites was applied to examine the mechanisms of cation formation, showing the determining role of optical and thermal properties on ion generation and suggesting that gas-phase processes determine the sodium adduction distribution.
- The wavelength dependent response of the mass spectra shows that the ion yield and species depends strongly on the density of vibrational excitation, most likely due to the operative mechanisms of ablation.

- Residual water incorporated during crystallization proves to be a sufficient chromophore to initiate laser ablation and allow cation formation.
 - The initial pH of the system did not generally affect the cations observed, either with respect to ion intensity or salt formation, suggesting that models based on ‘precharged’ associations are not operative for this system.
 - The sodium adduction patterns suggested that under conditions of high vibrational excitation adduction occurred under equilibrium conditions, while under moderate excitation the adduction was likely kinetically limited. Recent computational modeling of sodium affinities seems to support the adduction patterns.
 - Irradiation of an intrinsic component of the sample as a matrix represents a new paradigm for analytical mass spectrometry using infrared lasers, providing that a suitable ionizing agent is present.
3. The wavelength dependent response of infrared matrix materials was probed with regard to ions generated, ablation damage, and new vibrational modes.
- Excitation at wavelengths near water-absorbing modes show a distinctive blueshift of the threshold energy with respect to the bulk IR absorption profile, while excitation away from water modes shows no shift, but an undetermined relationship to analyte ion generation. The overlap of intermolecular vibrational modes with the water modes, however, prevents a conclusive determination of the causative factor.
 - The ‘quality’ of the spectra depends on the specific vibrational mode targeted and appears not to be directly correlated with the bulk absorption cross section.
 - Laser damage at wavelengths not related to the bulk absorption show extensive thermal damage, pointing to the role of either incorporated solvent, high defect density, or altered absorption profile by damage from the leading edge of the laser pulse.

1.3 Dissertation epitome

The purpose of this work is to further understand the critical parameters responsible for ablation and ion formation in MALDI, and particularly in IR-MALDI. To this end the primary models of ion formation have been tested for several material systems. Models representing the current understanding of MALDI processes will be discussed in the context of the specific experiments. Chapter 3, which describes the collaborative work with researchers at Pacific Northwest National Laboratory compares UV- and IR-MALDI for a variety of laser parameters. This published work [5] contains a review of the models of ion formation resulting from direct laser interaction with matrix molecules. Chapter 4, which describes work in the final stages of preparation for submission, includes a discussion of the other two generally recognized models of ion formation; (1) the cluster ionization mechanism, which posits that the charge associations formed in the sample solution are maintained in the condensed phase and throughout the ablation event, and (2) secondary ion formation, which describes the observed ions as resulting from thermodynamic equilibrium processes in the expanding plume. The mechanisms of material removal in MALDI to date have focused primarily on the processes active in UV-MALDI, but the progress made to date in understanding IR-MALDI, explained in context of critical time and length scales used to describe ablation processes, are discussed in Chapter 5. Finally, Chapter 6 summarizes the results of the experimental work and discusses their impact on the understanding of IR-MALDI mechanisms.

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