

CHAPTER VI

SUMMARY AND CONCLUSIONS

The preceding chapters have demonstrated the progress made to better understanding the underlying mechanisms of MALDI, especially those operative with infrared lasers. The studies described in this work take advantage of a unique infrared light source that allowed the exploration of previously uninvestigated vibrational modes and excitation densities, plus other laser systems that allowed us to compare the importance of pulse duration and electronic excitation in the ultraviolet. Given that models of the operative mechanisms are more mature for UV-MALDI, a useful point of departure for describing IR-MALDI is to investigate the significant differences between the two processes, highlighting the unique aspects resulting from the use of infrared irradiation. This allows one to challenge the models presently known to describe MALDI and to develop new ones as it becomes appropriate.

Generally, the principal differences related to the different wavelengths are (1) the excitation processes (electronic vs vibrational), (2) the pool of potential matrices and analytes that can be used and (3) the volume of material removed per laser shot. The studies described in the previous chapters address these points, and additional commentary on those results with respect to the impact made on the field of IR-MALDI is described below. The first difference, that of the different excitation processes, is necessitated by the significant differences in photon energies between the two regimes. The second difference is related to several roles the matrix assumes in MALDI – the absorption of laser energy leading to material removal, chemically compatible with the analyte and assistance in ion formation of the analyte. The final difference between ultraviolet and infrared lasers listed above, the differences in material removal per laser shot, is recognized as a limitation in IR-MALDI, but not often commented on with regard to mechanisms. From fundamental principles, we know that the wavelength-dependent penetration depth determines the degree of electronic or vibrational excitation in the sample, which then defines the operative ablation mechanisms, resulting in the ejection of a wide range of products from molecular species to large clusters.

Chapter 3 of this work described experiments probing potential differences in mass spectra caused by various irradiation parameters. Previous studies had noted that similar spectra

could be obtained with different laser sources, but had not systematically investigated both a wide range of photon energies and pulse durations. Our experimental approach, with the most extensive range of excitation conditions yet studied, necessarily created vastly different charged species and densities, yet our results showed qualitatively similar spectra could be obtained in all cases. We therefore concluded that the similarity of mass spectra alone offers a poor or incomplete picture from which to determine the operative mechanisms for ion formation. A plausible model that could explain the apparent similarity in mass spectra would be ion formation by secondary plume processes, where regardless of the initial ion species created by primary excitation, the observed ions are determined by conditions of thermodynamic equilibrium.

The challenge left to the MALDI community attempting to understand mechanisms is then to either (1) investigate those parameters such as thermal, optical, or excitation properties that influence the pathways leading to molecular ejection and ion formation, or (2) develop new probes of the early stages of phase transition from the condensed to the gas phase. Since our paper describing our results mentioned in the preceding paragraph, it seems that the community concerned with fundamental mechanisms has adopted the latter approach, and increased attention has been placed on the importance of other observables, such as the initial ion velocity and thermodynamic properties of matrices. The remaining studies described in this work, however, adopted the first approach, choosing instead to target previously unexplored vibrational modes, unconventional matrices, and to look at the wavelength dependent response scanning across various absorption modes.

The second set of experiments, detailed in Chapter 4 and describing the identification of small polycarboxylic acids from sodium nitrate, relates important contributions for both analytical and fundamental perspectives. From an analytical point of view, the work describes a radically new approach to the study of complex systems made possible by tunable light sources. The ability to tune into an intrinsic vibrational mode of the sample, eliminating the need for addition of a matrix that may have undesirable chemical reactions with the analyte, simplifies the sample preparation protocol and extends the MALDI technique to new classes of material systems. Given the success of compound identification when exciting water vibrational modes, which are accessible with fixed frequency lasers, it seems that this approach is extendable to many potentially interesting systems, given the ubiquity of water in many systems, providing

that a suitable ionizing agent is present. For the specific problem motivating these studies, the hazardous waste sludge resulting from nuclear fuel processing, we also demonstrated that the wide range of pH values is not likely to pose a problem for speciation of the organic components.

From a mechanistic point of view, the optical and thermal properties of the sample were found to determine the ablation pathway, and thus the ion species. Because the penetration depths in UV-MALDI experiments (~10-100 nm) and pulse durations (nanoseconds) are fairly similar, material removal is not considered an important parameter in ionization mechanisms, as the combination of matrices and wavelengths available to them are not likely to result in different ablation pathways. However, in the infrared, where penetration depths are an order of magnitude longer, resulting in a much larger amount of material removed per laser shot, the number and size density of the ejected material may have important consequences on the ion formation process, and can particularly address models relying on cluster ionization. While the studies recorded here did not directly measure the plume components, they were comparable to similar systems where the ablation processes have been better studied, and allowed us to make reasonable assumptions about the operative mechanisms. We found that the shorter penetration depths, with values similar to those found for ultraviolet laser-matrix combinations, resulted in an efficient reduction of the sample to molecular and fragment species, and generally good shot-to-shot reproducibility. This efficient disintegration created a surfeit of free sodium ion that were available to act as an ionizing agent. However, irradiation at wavelengths with longer penetration depths resulted in poorer shot-to-shot reproducibility without the high yield of matrix ions. Additionally, the analyte ion species observed showed a reduced yield of free sodium ions and significant differences in sodium adduction. Profilometry and microscopy of laser damage to single crystals revealed thermal damage to single crystals at short penetration depths, and fracture or explosive processes at longer penetration depths. These results confirm that the optical and thermal properties of the material strongly determine the material response, which subsequently determines the ion formation process. Practically, these results suggest that when selecting compounds in a mixture as potential matrices, the optical properties of the compounds should be carefully considered.

From a more fundamental perspective, the studies of the polycarboxylic compounds in sodium nitrate allow us to make some statements as to the applicability of certain models of ion formation for this system. Studies of samples prepared at different pH values and consideration

of the sodium adduction patterns suggests that the observed ions are not likely formed prior to irradiation, as has been posited by a number of authors, but rather in the expanding plume. The results from irradiation wavelength studies additionally suggest that abundant sodium ions are formed under conditions of high excitation density, allowing for ion formation to occur in thermodynamic equilibrium. This appears to be validated by our recent calculations of sodium binding affinities, which at least qualitatively agree with the sodium adduction patterns observed. Refinements to these calculations should allow us to model the reaction kinetics for gas-phase binding of sodium atoms, and determine if the adduction patterns indeed describe equilibrium conditions.

The final chapter describes observations made in targeting vibrational modes with different absorption cross-sections. When scanning the irradiation wavelength across an absorption mode and monitoring the energy required to see ion signal, one finds that in contrast to the results seen for sodium nitrate, the ion production response did not match well with the bulk absorption cross-section. If the primary condition for detecting ions is to effect a transfer of material into the gas phase, and if the number of ions scales roughly with the volume of material removed, at least near threshold, then the absorption cross section should scale with the energy required to see analyte ions - or, more carefully, with the inverse of the energy values. However, in a several experiments, in accord with studies from other research groups, both the minimum energy threshold and the 'best' spectra are obtained when wavelengths shorter than the absorption peak are used. This effect has been noticed only near absorption modes that overlap water modes and only for modes of ligands involved in intermolecular binding. For wavelengths targeting other absorption modes, the correlation between the inverse energy and absorption cross-section is better, although the quality of the spectra is poor. The studies described here included observations of laser damage to single crystals at different wavelengths and targeting of previously unexplored vibrational modes. When targeting these 'favorable' wavelengths, which have a very low bulk absorption cross-section but still result in the most favorable and efficient spectra, the laser damaged surface shows evidence of extensive melting. This is somewhat surprising, for based on ablation models for these wavelengths and optical properties, material removal should be initiated deep in the bulk and non-thermal mechanisms should dominate. These results points to the role of increased absorption either due to residual solvent incorporated in the crystals or the increased absorption due to laser damaged bonds. The latter is in agreement

with a hypothesis previously put forward to explain this wavelength-shift phenomenon, that the early part of the laser pulse weakens the intermolecular bonds, which in turn shifts the absorption profile to higher frequencies, resulting in a higher absorption profile at that wavelength. These observations suggest that the bulk absorption profile is a poor indicator of the changing cross-section that exists during irradiation. Also, it must be remembered that one is observing only a minority fraction of the total ablated material, and so there is strong dependence on those parameters involved in ion formation.

In summary, the primary contributions of this work show: (1) that the critical ion formation stage in IR-MALDI likely occurs in the expanding plume and not in the solution phase or during irradiation, and ion formation is kinetically limited when the vibrational excitation density is low and determined by thermodynamic equilibrium when the excitation density is high, (2) that excitation of intrinsic vibrational modes is a promising new protocol for IR-MALDI, and (3) that while the optical and thermal properties of the matrix are necessary determinants of the ablation pathways, alone they are insufficient to predict the efficiency of ion production in IR-MALDI.