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**Design, Construction and Development of a Laser Desorption
Ionization/Laser Ablation Time-of-Flight Mass Spectrometer for
Chemical Analysis with and without Surface Plasmon Resonance**

By

Sandy Owega

A Dissertation submitted to the Faculty of Graduate Studies in partial fulfilment of the
requirements for the degree of Doctor of Philosophy

Department of Chemistry

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**Design, Construction and Development of a Laser Desorption Ionization/
Laser Ablation Time-of-Flight Mass Spectrometer for Chemical Analysis
with and without Surface Plasmon Resonance**

Submitted by
Sandy Owega, B.Sc.

in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

Chair, Department of Chemistry

Thesis Supervisor

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Abstract

Theoretical modeling of the Wiley-McLaren double-focusing field system (two acceleration fields) provided the critical dimensions for the design and construction of a time-of-flight mass spectrometer (TOFMS) for this research. For optimum resolution, the distances within the acceleration fields, s (0.26 cm) and d (2.60 cm) were determined for a drift tube length D of 42.2 cm. Arcing occurred frequently using our laser desorption ionization (LDI)/laser ablation (LA) technique, five different configurations were designed and evaluated. The third configuration was determined to be the most useful for LDI/LA-TOFMS experiments.

The LDI/LA technique was tested for molecular mass and structural reactivity analysis. A sample was deposited by solvent evaporation on the thin silver film and subjected to a 355-nm or 532-nm laser beam from a Nd-Hyper YAG laser via back-illumination. Silver cluster cations $[\text{Ag}_n]^+$ ($1 \leq n \leq 3$) were primarily observed in the mass spectrum and conveniently used to calibrate the TOFMS spectrum. This LDI/LA technique was successfully applied to dithizone, 1,4,8,11-tetraazocyclotetradecane, dicyclohexyl-18-crown-6 ether, [5]-helicene dendrimer, gramicidin S, substance P, mellitin, PAHs, fullerenes/derivatives, thia fatty esters/acids, and a variety of related compounds. Gas-phase cationization by Ag^+ was fully verified by the appearance of a $[\text{M}+\text{Ag}]^+$ cation, although the structure of the neutral compound dictated its production. In general, gas-phase reactions occurred with compounds that possess electron rich sites on their functional groups. One advantage of the present LDI/LA technique over

conventional ones is that the sample does not need to have appreciable spectral absorption at the laser wavelength.

The physical process that occurred during our LDI/LA technique was elucidated with internal standardization and ion association using gramicidin S. The samples reacted in the gas phase to produce M^+ , $[M+Ag]^+$ and/or $[M+Na]^+$ cations. The cations and fragments were thought to be the products of electron-transfer reactions. With increasing silver film thickness, the $[M+Ag]^+/Ag^+$ ratio decreased, but a maximum $[M+Ag]^+$ cation signal was observed for a silver film thickness with a spectrophotometric absorbance of 0.6 (equivalent to a thickness of approximately 20 nm). The LDI/LA mechanism generating the $[M+Ag]^+$ cation was thought to be electronic-excitation (at low laser fluences) that evolved into a thermal one (at high laser fluences), depending on the silver film thickness.

The five configurations were also evaluated for incorporating surface plasmon resonance (SPR) into our LDI/LA technique to ultimately construct a novel SPR-LDI/LA-TOFMS instrument. The fifth configuration was determined to be good for SPR-LDI/LA-TOFMS experiments. Theoretical modeling of the SPR phenomenon was in agreement with experimental results. They indicated that silver surface plasmons have a SPR angle θ_r of 44° and an energy of 3.7 eV for a thin silver film thickness of 40 nm. The SPR-LDI/LA technique demonstrated that a lower minimum laser fluence for the production of the silver cluster cations $[Ag_n]^+$ was required at θ_r . Even when gramicidin S was deposited on the surface of the thin silver film, the $[M+Ag]^+$ and/or $[M+Na]^+$ cations were observed at a lower minimum laser fluence at the θ_r . SPR was thus

confirmed to assist in the electronic-excitation desorption during LDI/LA of a thin silver film with or without deposited samples. The capability to perform SPR-LDI/LA on a molecular weight of 1141 Da from a thin silver film represents a new milestone beyond previous achievements. Furthermore, the effect of matrix isolation was observed when 2,5 dihydroxybenzoic acid was applied on the silver film prior to deposition of gramicidin S, and an even lower laser fluence was required to produce a $[M+Na]^+$ cation at the θ .

Publications

1. Owega, S.; Lai, E.P.C.; Bawagan, A.D.O. "Surface-Plasmon Resonance (SPR) - Laser Desorption/Ionization (LDI) - Time-of-Flight Mass Spectrometry (TOFMS)" *Anal. Chem.* 1998 JUN 1 Vol.70 No.11 Pages 2360 - 2365.
2. Lai, E.P.C.; Owega, S.; Kulczycki, R. "Time-of-Flight Mass Spectrometry of Bioorganic Molecules by Laser-Ablation of Silver Thin Film Substrates and Particles" *J. Mass Spectrom.* 1998 JUN Vol.33 No.6 Pages 554-564.
3. Owega, S.; Lai, E.P.C.; Mullett, W. "Laser Desorption Ionization of Gramicidin S on Thin Silver Films with Matrix Isolation in Surface Plasmon Resonance Excitation" *J. Photochemistry and Photobiology A: Chemistry* 1998 DEC Vol.119 No.2 Pages 123 - 135.
4. Owega, S.; Lai, E.P.C. "Silver Cationization of Thia Fatty Acids and Esters in Laser Desorption Ionization Time-of-Flight Mass Spectrometry" *J. Mass Spectrom.* 1999 AUG Vol.34 No.8 Pages 872 - 879.

Conference Presentations

1. 82nd Canadian Society for Chemistry Conference and Exhibition, Toronto, Ontario, Canada. May30-June2, 1999. "Laser Desorption Ionization of Gramicidin S on Thin Silver Films with Matrix Isolation in Surface Plasmon Resonance Excitation." S. Owega and E.P.C. Lai.
2. Varian Day Lecture, Carleton University, Ottawa, ON, Canada. April 1999. "Laser Desorption Ionization of Gramicidin S on Thin Silver Films with Matrix Isolation in Surface Plasmon Resonance Excitation." Owega, S. and Lai, E.P.C.
3. 81st Canadian Society for Chemistry Conference and Exhibition, Simon Fraser University, Whistler, BC, Canada. May31-June 1, 1998. "Surface Plasmon Enhanced Laser Desorption Ionization Mass Spectrometry of Bioorganic Compounds." S. Owega and Lai, E.P.C.
4. Varian Day Lecture, Carleton University, Ottawa, ON, Canada. April 14, 1998. "Surface Plasmon Resonance (SPR) Laser Desorption Ionization (LDI) Time-of-Flight Mass Spectrometry (TOFMS)." Owega, S. and Lai, E.P.C.

Poster Presentations

1. 82nd Canadian Society for Chemistry Conference and Exhibition, Toronto, Ontario, Canada. May 30-June 2, 1999. "Silver Cationization of Thia Fatty Acids and Esters in Laser Desorption Ionization Time-of-Flight Mass Spectrometry." S. Owega and Lai, E.P.C.
2. Ottawa-Carleton Chemistry Institute Symposium, Ottawa, ON, Canada. May 1999. "Silver Cationization of Thia Fatty Acids and Esters in Laser Desorption Ionization Time-of-Flight Mass Spectrometry." Owega, S. and Lai, E.P.C.
3. 81st Canadian Society for Chemistry Conference and Exhibition, Simon Fraser University, Whistler, BC, Canada. May 31-June 4, 1998. "Capillary Electrochromatography of Phospholipids with Photodiode Array and Laser Desorption Ionization Time-of-Flight Mass Spectrometry Detections." E.P.C. Lai and S. Owega; E. Dabek- Zlotorzynska.
4. Ottawa-Carleton Chemistry Institute Symposium, Ottawa, ON, Canada. May 1998. "Surface Plasmon Resonance (SPR) Laser Desorption Ionization (LDI) Time-of-Flight Mass Spectrometry (TOFMS)." Owega, S. and Lai, E.P.C.
5. 80th Canadian Society for Chemistry Conference and Exhibition, Windsor, Ontario, Canada. June 1-4, 1997. "The Study of Peptides using SPR-LDI-TOFMS." Owega, S. and Lai, E.P.C.
6. Ottawa-Carleton Chemistry Institute Symposium, Ottawa, ON, Canada. May 1997. "Formation of Ag_n^+ Clusters by Photoablation of a Silver Film." Owega, S. and Lai, E.P.C.
7. 79th Canadian Society for Chemistry Conference and Exhibition, St. John's, Newfoundland, Canada. May-June, 1996. "Surface Plasmon Resonance Laser Desorption Ionization for Time-of-Flight Mass Spectrometry." Owega, S. and Lai, E.P.C.
8. Ottawa-Carleton Chemistry Institute Symposium, Ottawa, ON, Canada. May 1997. "Surface Plasmon Resonance (SPR) - Laser Desorption Ionization (LDI) - Time-of-Flight Mass Spectrometry (TOFMS)" Owega, S. and Lai, E.P.C.

Acknowledgments

There are so many individuals who deserve the utmost thanks in aiding me not only with this report, but in supporting me throughout this very long time at Carleton University (roughly 10 to 12 years – I’ve lost count). Dr. E.P.C. Lai, you always believed in my ability and offered guidance, reason and understanding. I am very grateful that you had a lot of patience with my writing and my ideas. I would also like to thank my colleague, Wayne Mullett for his support, Fred Cassalman for making this place bearable, and the Science Technology Center (STC) for constructing items for this project. I would also like to acknowledge Dr. N. DeSilva and Dr. A.D O. Bawagan for their help.

Lastly, I would like to thank my family, but most of all, my wife, Sylvia for their encouragement. I know that without them, I would have been miserable.

If I have missed out anyone else, my sincere apologies.

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List of Symbols

Symbol	Description
SPR	surface plasmon resonance
LDI	laser desorption ionization
LA	laser ablation
TOFMS	time-of-flight mass spectrometer
MALDI	matrix-assisted laser desorption ionization
SEM	scanning electron microscopy
ESI	electrospray ionization
CI	chemical ionization
ATR	attenuated total reflection
LED	light emitting diode
PDA	photodiode array
PAS	photoacoustic spectroscopy
DSO	digital storage oscilloscope
MCP	micro-channel plate
FWHM	full width at half maximum
PAH	polyaromatic hydrocarbon
CRF	charge remote fragmentation
DHB	2,5-dihydrobenzoic acid
HIV	human immunodeficiency virus
E	electric field strength

B	magnetic flux density
J	total current density
P	electric polarization (or dipole moment per unit volume)
D	electric flux density (or electric displacement)
x	displacement
k	wave vector
EM	electro-magnetic
θ_r	SPR angle
R	reflectance (or reflected light intensity)
q_e	charge of electron (1.6022×10^{-19} Coulombs)
m_e	mass of electron (9.1×10^{-31} kg)
m/z	mass-to-charge ratio
c	speed of light (3×10^8 m/s)
t	time
v	velocity
s	distance between grid A and grid B
d	distance between grid B and grid C
D	distance of field-free drift tube (or distance between grid C and grid D)
ϵ	permittivity of medium
μ	permeability of medium
n	refractive index of medium
ρ	total electric charge density

ω frequency

Chapter 1: Introduction

1.1 General Introduction

The detection of intact macromolecules in mass spectrometry is attracting a great interest in science. New ionization techniques have been introduced, like atmospheric pressure ionization (Sin et al., 1991), pulsed sample introduction (Wang and Li., 1992), and plasma source atmospheric pressure ionization (Chien et. al., 1993), but the general results have indicated thermal breakdown, and hence fragmentation of the macromolecule before being detected. Fragmentation of the macromolecule is the result of a hard ionization process. Two softer ionization processes that have been recently developed are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). The ESI technique generates intact multiply charged sample molecules and is easily coupled to liquid separation techniques. A quadrupole mass spectrometer is commonly chosen to detect the continuous stream of sample molecular ions generated by the ESI method. The MALDI technique generates intact sample molecular ions from the solid phase with a short laser pulse and is easily adapted to time-of-flight mass spectrometers (TOFMS). The large upper mass limit of TOFMS instruments enables the detection of large singly charged molecular ions. In fact, intact poly(styrene) ions of molecular weight 1.5 MDa have been successfully detected using the MALDI-TOFMS instrument (Schriemer and Li, 1996).

MALDI was first recognized by Michael Karas and Franz Hillenkamp (Karas and Hillenkamp, 1989). They observed that non-volatile amino acids and peptides mixed

with particular MALDI matrix compounds reacted in the gas phase within an ablation plume during the LDI process. Absorption of the laser light by the MALDI matrix must occur (i.e. the MALDI matrix has a chromophore at the laser wavelength (Vertes et al., 1993)), of which the more successful MALDI matrices have hydroxy substituted in the para position and methoxy substituents on the ring (Carr and Brown, 1995). The sample is protonated by the MALDI matrix without fragmenting significantly. Two of the limitations of MALDI are the precise ratio of matrix to analyte (Beeson et al., 1995) and the specific MALDI matrix that must be chosen for an analyte (Sze et al., 1998). If an unknown sample is analyzed with the MALDI technique, these limitations can increase analysis time and expenses. A minor disadvantage is that MALDI matrix fragment ions can interfere with sample molecular fragment ions. Clearly, TOFMS analysis of low molecular weight samples is better performed with a different ionization technique that does not use a matrix (Wingerath et al., 1997).

The cationization of bio-organic compounds was first demonstrated as an alternative approach to ionize solid samples during the pre-MALDI era (Posthumus et al., 1978). This technique, in fact, could alleviate some of the limitations of MALDI. Metal cations would behave as chemical ionization (CI) reagents that ionize bio-organic compounds in the gas phase within an ablation plume during the LDI process (Burnier et al., 1981). Depending on the electronic nature of the metal cation, different types of reactions are promoted. For example, transition metals may insert into covalent bonds of organic molecules, whereas alkali and alkaline earth metals interact through electrostatic interactions (Wu and Brodbelt, 1994). The cationization of benzene with gold cations (Louris et al., 1985), hydrocarbon polymers with silver cations (Kahr and Wilkins, 1993),

and acetone with chromium, nickel, iron and copper cations (Gill et al., 1996) are only a few of the gas-phase reactions that were reported occurring in the LDI process. Similar to the MALDI technique, however, the cationization reaction is known to be analyte dependent. In fact, the role of d-electrons of the cation-molecule chemical bond is still a challenging problem for experimentalists and theoreticians (Hachimi and Muller, 1997).

Improvement on the detection of intact macromolecules was accomplished by performing the LDI technique on thin organic films (Speir and Amster, 1992). This concept was further developed to include stainless steel and other metal films (Knowles and Leone, 1997; Brunco et al., 1993). This LDI technique decreased the internal energy of the analyte molecules. Consequently, the parent ion was easily detected in the mass spectrum because fragmentation was reduced by an order of magnitude (Zhan et al., 1997). Depending on the electronic nature of the thin metal film, the sample was either present or absent from the LDI-TOFMS spectrum (Bjarnason, 1996). As previously mentioned, the gas-phase reaction between the metal cation and the sample molecule is specific. Since optical phenomena are governed by the electronic nature of thin metal films, surface plasmon resonance (SPR), described in Section 1.2, should influence the observation of an intact molecular cation in a mass spectrum. During SPR, the thin metal film absorbs the laser light and transfers this energy to assist in the LDI process of the analyte. Consequently the threshold laser fluence is reduced, as demonstrated by the successful detection of intact rhodamine B dye molecules undergoing the SPR-LDI process using a thin aluminum film (Lee et al., 1992). The fragmentation of the intact cationized molecular ion is reduced and its detection is enhanced. In fact, the threshold laser fluence required for laser ablation (LA) of a thin aluminum film under SPR

conditions was reduced 3-5 times compared to LA without SPR conditions (Dawson and Cairns, 1994).

In this work, an SPR-LDI/LA-TOFMS instrument was built to analyze bio-organic compounds. Modeling of the SPR phenomenon and optimization of the dimensions for the TOFMS were performed. Since silver exhibited the SPR phenomenon and was known to be an effective CI reagent for bio-organic compounds, it was the metal of choice. The silver cationizing power was evaluated by changing the molecular structure and electron withdrawing/donating power of functional groups of bio-organic compounds. Experiments with different LDI/LA-TOFMS instrumental configurations were performed to elucidate the cationization mechanism with and without SPR conditions. Only optimized configurations were able to successfully detect intact bio-organic compounds. The LDI/LA process of bio-organic compounds was evaluated, whether a chromophore in the bio-organic compound was present or not. The potential benefit of the SPR-LDI/LA-TOFMS instrument was evaluated by optimizing a technique with select attributes (analyte-dependent efficiencies) that may serve for the direct and accurate mass analysis of significant bio-organic compounds.

1.2 Surface Plasmon Resonance (SPR)

1.2.1 Generation and Energy of the Silver Surface Plasmon

SPR is an optical phenomenon that allows a thin metal film to absorb p-polarized light at a specific angle of incidence. By the conservation of energy (Newton's law of motion), this energy can be used to assist our LDI/LA technique. Knowing the electric and magnetic properties of the chosen metal, and understand the characteristic features of light (electromagnetic (EM) waves), an equation is derived below to calculate the quantity of energy absorbed by the thin metal film.

The nature of EM waves is described through the Maxwell equations (Lorrain et al., 1988):

$$\nabla \cdot \mathbf{E} = \frac{\rho}{\epsilon_0} \quad 1.2.1-1$$

$$\nabla \times \mathbf{E} + \frac{\partial \mathbf{B}}{\partial t} = 0 \quad 1.2.1-2$$

$$\nabla \cdot \mathbf{B} = 0 \quad 1.2.1-3$$

$$\nabla \times \mathbf{B} - \frac{1}{c^2} \frac{\partial \mathbf{E}}{\partial t} = \mu_0 \mathbf{J} \quad 1.2.1-4$$

The variables in these equations are defined as follows:

$\mathbf{E}$ is the electric field strength;

ρ is the total electric charge density;

ϵ_0 is the permittivity of free space ($\sim 8.85 \times 10^{-12}$ farad/meter);

$\mathbf{B}$ is the magnetic flux density;

t is time;

c is the speed of light (3×10^8 m/s);

μ_0 is permeability of free space;

$\mathbf{J}$ is the total current density.

These equations assume that the EM wave is traveling through a homogeneous, linear and isotropic medium. These equations apply to any number or diversity of sources.

The first Maxwell equation indicates that the divergence of an electric field $\mathbf{E}$ is related to the total electric charge density ρ . However, in dielectric media, the total charge density is the sum of the free and the bound charge densities. The bound charge density ρ_b is described by the negative divergence of the dipole moment per unit volume or the electric polarization $\mathbf{P}$:

$$\rho_b = -\nabla \cdot \mathbf{P} \quad 1.2.1-5$$

Dielectric media are linear and isotropic when the electric polarization $\mathbf{P}$ is proportional to the applied electric field $\mathbf{E}$ by the electric susceptibility. If the electric susceptibility is independent of the coordinates, the dielectric will be homogeneous. The electric polarization $\mathbf{P}$ can also be represented by the sum of all the contributing dipole moments from N electrons per unit volume. The product of the charge q_e of an electron and its displacement $\mathbf{x}$ is defined as the dipole moment, therefore the electric polarization is written as:

$$\mathbf{P} = q_e \mathbf{x} N \quad 1.2.1-6$$

The free charge density ρ_f is the divergence of the electric flux density, also called the electric displacement $\mathbf{D}$:

$$\rho_f = \nabla \cdot \mathbf{D} \quad 1.2.1-7$$

The electric displacement $\mathbf{D}$ within a linear material can be expressed as a function of the applied electric field $\mathbf{E}$ and the permittivity ϵ :

$$\mathbf{D} = \epsilon \mathbf{E} \quad 1.2.1-8$$

It is usually written as a function of the applied electric field and the electric polarization:

$$\mathbf{D} = \epsilon_0 \mathbf{E} + \mathbf{P} \quad 1.2.1-9$$

When light interacts with matter, the dependence of the refractive index n on the frequency (and the wavelength) ω is governed by the electric polarization mechanisms contributed by that frequency.

Conducting atoms can be viewed as a series of damped oscillators with each atom consisting of a positive nucleus surrounded by an electron cloud (Hecht, 1987). An electric force sustains the equilibrium between these charged particles. To simplify the derivation, only the x-coordinate is considered. When a time-dependent electric field of a lightwave $E(t)$ is imposed on an atom, a restoring force F_x between the electron cloud and the positive nucleus exists:

$$F_x = -kx \quad 1.2.1-10$$

The electric field of the lightwave will also contribute a force:

$$F_x = q_e E(t) = q_e E_0 \cos \omega t \quad 1.2.1-11$$

As a consequence of the external force, the electron will oscillate about its equilibrium position with a resonant frequency ω_0 :

$$\omega_0 = \sqrt{k/m_e} \quad 1.2.1-12$$

The relationship between the restoring force and the electric force of the lightwave is

obtained when applying Newton's second law:

$$q_e E_0 \cos \omega t - m_e \omega_p^2 x = m_e \frac{d^2 x(t)}{dt^2} \quad 1.2.1-13$$

By assuming the electron oscillation will have the same frequency as the lightwave, the equation for $x(t)$ has a similar form:

$$x(t) = x_0 \cos \omega t \quad 1.2.1-14$$

Hence, the solution for $x(t)$ is determined:

$$x(t) = \frac{q_e / m_e}{(\omega_p^2 - \omega^2)} E(t) \quad 1.2.1-15$$

This relationship describes a lightwave interacting with an atom to cause the relative displacement between the electron cloud and the positive nucleus of the atom to oscillate, forming a dipole.

By substituting $x(t)$ into Eq. 1.2.1-6 for calculating the electric polarization P , the following relationship is attained:

$$P = \frac{q_e^2 N E / m_e}{(\omega_p^2 - \omega^2)} \quad 1.2.1-16$$

Furthermore, substituting the electric polarization P into Eq. 1.2.1-9 gives the relationship between the permittivity ϵ and the frequency of the lightwave ω :

$$\epsilon = \epsilon_0 + \frac{q_e^2 N / m_e}{(\omega_p^2 - \omega^2)} \quad 1.2.1-17$$

By definition, the electric permittivity of a material, ϵ is the product of its relative electric permittivity, ϵ_r and the electric permittivity in free space, ϵ_0 . The Maxwell relation can be used to express the refractive index n of a material in terms of the relative permittivity

of a material $\epsilon_r = \epsilon' - i\epsilon''$.

$$n = \sqrt{\epsilon_r} \quad 1.2.1-18$$

Now, a dispersion relationship describing the refractive index n as a function of the frequency ω of the lightwave is determined:

$$n^2 = 1 + \frac{q_e^2 N}{\epsilon_0 m_e (\omega_0^2 - \omega^2)} \quad 1.2.1-19$$

This equation can be written in a more general form, which considers more than one oscillator f having a natural frequency ω_0 :

$$n^2(\omega) = 1 + \frac{Nq_e^2}{\epsilon_0 m_e} \sum_f \frac{f_f}{\omega_{0f}^2 - \omega^2} \quad 1.2.1-20$$

The f_f terms are weighting factors known as oscillator strengths, or transition probabilities, that dictate the emphasis placed on each mode. The sum of these weighting factors is one. To describe the energy lost when the forced oscillators reradiate in the form of heat, an additional imaginary term $i\gamma_f \omega$ is included in the denominator of the sum. To involve the contributions given by the free electrons, an additional term must be added to give the final form of the equation:

$$n^2(\omega) = 1 + \frac{Nq_e^2}{\epsilon_0 m_e} \left[\frac{f_e}{-\omega^2 + i\gamma_e \omega} + \sum_f \frac{f_f}{\omega_{0f}^2 - \omega^2 + i\gamma_f \omega} \right] \quad 1.2.1-21$$

Notice that the free electrons have a zero restoring force, while the tightly bound electrons have a restoring force.

For metals, the conduction electrons (or free electrons) are the predominant contributors to the optical properties. Bearing this in mind, we can neglect the contribution given by the bound electrons (the summation term is set to zero). We also

assume that the free electrons in a metal will oscillate many times, without damping, at very large ω (the $i\gamma_e\omega$ is set to zero). This transforms the equation to give:

$$n^2(\omega) = 1 - \frac{Nq_e^2}{\epsilon_0 m_e \omega^2} \quad 1.2.1-22$$

The free electrons and positive ions within a metal can be viewed as a bulk plasma density that oscillates at a natural frequency ω_p . The bulk plasma frequency ω_p in Eq. 1.2.1-22 is defined as:

$$\omega_p^2 = \frac{Nq_e^2}{\epsilon_0 m_e} \quad 1.2.1-23$$

Now, the refractive index n can be written as a function of the frequency of the incident EM wave:

$$n^2(\omega) = 1 - (\omega_p / \omega)^2 \quad 1.2.1-24$$

Identically, the relationship between the relative permittivity of the conductor and the EM frequency is:

$$\epsilon_r = 1 - (\omega_p / \omega)^2 \quad 1.2.1-25$$

Consider a semi-infinite plasma in the conducting metal existing on the positive side of a plane ($z=0$), and air existing on the negative side of the plane. Poisson's equation relates the total electric charge density ρ at a given point to the second space derivatives of the voltage V in the region of that point (Kittel, 1976):

$$\nabla^2 V = -\frac{\rho}{\epsilon_0} \quad 1.2.1-26$$

Since the total charge density ρ at the interface between the conducting medium and the vacuum medium is zero, Laplace's equation is formed:

$$\nabla^2 V = 0 \quad 1.2.1-27$$

The solution of Laplace's equation in the air medium (i.e. $z < 0$) for a decaying wave (or an evanescent wave) is:

$$V(x, z) = A \cos(kx) e^{kz} \quad 1.2.1-28$$

Similarly, the solution of Laplace's equation in the conducting metal (i.e. $z > 0$) for a decaying wave is:

$$V(x, z) = A \cos(kx) e^{-kz} \quad 1.2.1-29$$

The electric field in the air medium is determined to be:

$$\begin{aligned} \mathbf{E} &= -\nabla V \\ &= Ak \sin(kx) e^{kz} \hat{\mathbf{x}} - Ak \cos(kx) e^{kz} \hat{\mathbf{z}} \end{aligned} \quad 1.2.1-30$$

and the electric field in the conducting metal is determined to be:

$$\begin{aligned} \mathbf{E} &= -\nabla V \\ &= Ak \sin(kx) e^{-kz} \hat{\mathbf{x}} + Ak \cos(kx) e^{-kz} \hat{\mathbf{z}} \end{aligned} \quad 1.2.1-31$$

Since the energy of the EM wave traveling through the air medium is transferred to the conducting metal, the tangential component of $\mathbf{E}$ (or the x-component of the electric field) at the plane $z=0$ must be continuous. This means that E_x for both media must be the same at $z=0$ (i.e. at the plane).

Another requirement for energy conservation is that the normal component of $\mathbf{D}$ (or the z-component of the electric displacement) at the plane $z=0$ is continuous at the interface. To determine the effect of the EM wave on the electric displacement $\mathbf{D}$ within the conducting metal and the vacuum, recall Eq. 1.2.1-8. In air, the value of ϵ_r is 1. Now, the electric displacement $\mathbf{D}$ within the air medium is written:

$$\begin{aligned}
 \mathbf{D} &= \varepsilon A k \sin(kx) e^{kz} \hat{\mathbf{x}} - \varepsilon A k \cos(kx) e^{kz} \hat{\mathbf{z}} \\
 &= \varepsilon_0 A k \sin(kx) e^{kz} \hat{\mathbf{x}} - \varepsilon_0 A k \cos(kx) e^{kz} \hat{\mathbf{z}}
 \end{aligned}
 \tag{1.2.1-32}$$

The electric displacement $\mathbf{D}$ within the metal conductor is written:

$$\mathbf{D} = \varepsilon A k \sin(kx) e^{-kz} \hat{\mathbf{x}} + \varepsilon A k \cos(kx) e^{-kz} \hat{\mathbf{z}}
 \tag{1.2.1-33}$$

By equating the normal component of the electric displacement $\mathbf{D}$ for both media at $z=0$, the relative permittivity of the conducting metal is -1 . Substituting this dielectric value into Eq. 1.2.1-25, the relationship between the surface plasmon frequency ω_s and the bulk plasmon frequency is determined.

$$\omega_s^2 = \frac{1}{2} \omega_p^2
 \tag{1.2.1-34}$$

In this scenario, the EM waves generate surface plasmons (or electron plasma waves) that behave as transverse electromagnetic waves along the surface of a thin metal film. This phenomenon is called surface plasmon resonance, or SPR.

The chosen metal in this work is silver, which has a bulk plasmon energy of 9.2 eV. Using Eq. 1.2.1-34, the calculated surface plasmon frequency is 6.5 eV, which lies far above the experimental surface plasmon frequency of 3.7 eV (Liebsch, 1997). This discrepancy is a consequence of metal interband electronic transitions that alter the plasmon dispersion curve significantly. In general, the interband electronic transitions from the occupied d or s-p bands to higher-lying unoccupied s-p bands obscure the surface plasmon modes. This is true for the filled 4d bands of silver. However, it is the photon energy of 3.7 eV acquired by the thin silver film during SPR that will assist the LDI/LA process.

1.2.2 Angular Dependence of SPR on Wavelength

SPR can be achieved by exciting the thin silver film with light. The surface plasma waves are transverse and they oscillate along the plane (at $z=0$) at the air/silver boundary (Szentirmay, 1992; Welford, 1991). Also, the light propagates freely through the air medium, but attenuates rapidly through the silver film. However, to absorb light, momentum must be conserved. This is best expressed by deriving and equating the wave vectors for the light and surface plasmon oscillations. Wave vectors have a direction and are a function of the permittivity, and hence dielectric constant, of the silver film and air medium. Also, silver's dielectric constant changes with wavelength of light. To accommodate any choice of laser, a relationship between wavelength and incidence angle is first derived, as shown below.

The wave vector or direction of the light is determined by altering some of Maxwell's equations in the previous section for non-magnetic media. The most important aspect of this derivation lies in the assumptions that will be made about the media: homogeneous, linear, isotropic and stationary. Mathematically written:

$$\rho = \frac{\rho_f}{\epsilon_r} \quad 1.2.2-1$$

$$\mathbf{P} = \chi_e \epsilon_0 \mathbf{E} = (\epsilon_r - 1) \epsilon_0 \mathbf{E} \quad 1.2.2-2$$

These assumptions modify the Maxwell equations for dielectric media by using the mathematical relation:

$$\nabla \times \nabla \times \mathbf{E} = -\nabla^2 \mathbf{E} + \nabla(\nabla \cdot \mathbf{E}) \quad 1.2.2-3$$

Substituting the right-hand side of Eq. 1.2.2-3 into Eq. 1.2.1-2 and taking the curl of

$\partial \mathbf{B} / \partial t$ gives:

$$\nabla^2 \mathbf{E} - \nabla(\nabla \cdot \mathbf{E}) = \frac{\partial(\nabla \times \mathbf{B})}{\partial t} \quad 1.2.2-4$$

By substituting Eq. 1.2.1-4 into the left-hand side of Eq. 1.2.2-4, Eq. 1.2.2-5 is derived:

$$\nabla^2 \mathbf{E} - \nabla(\nabla \cdot \mathbf{E}) = \frac{\partial}{\partial t} (\mu \mathbf{J}_f + \epsilon \mu \frac{\partial \mathbf{E}}{\partial t}) \quad 1.2.2-5$$

By substituting Eq. 1.2.1-1 into the second term of the left-hand side of Eq. 1.2.2-5, the final form of the wave equation for the electric field is:

$$\nabla^2 \mathbf{E} - \epsilon \mu \frac{\partial^2 \mathbf{E}}{\partial t^2} = \frac{\nabla \rho_f}{\epsilon} + \mu \frac{\partial \mathbf{J}_f}{\partial t} \quad 1.2.2-6$$

In our case, the free charge density ρ_f does not alter in space and so $\nabla \rho_f$ must be zero. The current density $\mathbf{J}_f$ resulting from the motion of free charge does not change with time and so $\partial \mathbf{J}_f / \partial t$ must also be zero. Hence, the right-hand side of Eq. 1.2.2-6 is zero.

In free space, the relationship between the speed of light, c and the electric permittivity, ϵ_0 and magnetic permeability, μ_0 is given as:

$$c^2 = \frac{1}{\epsilon_0 \mu_0} \quad 1.2.2-7$$

By definition, the magnetic permeability of a material, μ , is the product of its relative magnetic permeability, μ_r , and the magnetic permeability in free space, μ_0 . For non-magnetic media, μ_r is 1. Thus, the final form of Eq. 1.2.2-6 can be written as:

$$\nabla^2 \mathbf{E} - \frac{\epsilon_r}{c^2} \frac{\partial^2 \mathbf{E}}{\partial t^2} = 0 \quad 1.2.2-8$$

A solution to Eq. 1.2.2-8 is:

$$\mathbf{E} = \mathbf{A}e^{i(k_x x - \omega t)}e^{ik_z z} \quad 1.2.2-9$$

Substitution of Eq. 1.2.2-9 into Eq. 1.2.2-8 gives an equation for the z-component of the wave vector, k_z :

$$k_z = (\varepsilon_r(\omega/c)^2 - k_x^2)^{1/2} \quad 1.2.2-10$$

In our scenario:

$$k_x > \sqrt{\varepsilon_r}(\omega/c) \quad 1.2.2-11$$

This result indicates that the z-component of the wave vector, k_z is imaginary. Accordingly, the electric field attenuates rapidly according to Eq. 1.2.2-9 and the thin silver film generates non-radiative surface plasmons. To excite the non-radiative surface plasmons, the momentum of the incoming photons, $\hbar k_z/2\pi$, must be increased. This is accomplished using the attenuated total reflection (ATR) method (Raether, 1977; Abeles, 1976), as described below.

Consider a thin silver film bound by two different media, as seen in Figure 1.1. If the incoming photons travel through a first medium with a refractive index, n , then the momentum of the photons will increase by $n\hbar k_z/2\pi$. The first medium in our experimental setup is a glass prism, which has a large refractive index of 1.515 and large relative permittivity of 2.295. By rearranging Eq. 1.2.2-10, the magnitude of the light wave vector can be written as:

$$k_x^2 + k_z^2 = k^2 = \varepsilon_r(\omega/c)^2 \quad 1.2.2-12$$

Figure 1.1 Attenuated total reflection method to generate non-radiative silver surface plasmons.

The relationship between the magnitude of the wave vector and the x-component of the wave vector is given by:

$$k \sin \theta = k_x \quad 1.2.2-13$$

The x-component of the wave vector of the light traveling through the glass prism is now written as:

$$k_{x,g} = \frac{\omega}{c} \sqrt{\varepsilon_{r,g}} \sin \theta \quad 1.2.2-14$$

Since plasmons are generated through the p-polarized light component along the metal surface, the angle of incidence θ must be introduced. The relationship between the wave vector k_x and the frequency ω is called a dispersion curve.

The thin silver film is now coupled to the glass prism with oil. To derive the dispersion curve for the wave vector of the silver surface plasmons as a function of the frequency ω , boundary conditions (identical to Chapter 1.2.1) must be enforced. These boundary conditions will be located at the silver/sample interface. The wave vector of the surface plasmon oscillations, K_{sp} , that describes the SPR phenomenon as a function of the frequency of oscillations ω , the speed of light c , the dielectric constant of the silver ε_1 , and the dielectric constant of the sample ε_2 is given by (Raether, 1977; Abeles, 1976):

$$K_{sp} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}} \quad 1.2.2-15$$

Since silver is a conductor, the dielectric constant (which is governed by its optical constants at a specific wavelength) must have a real and an imaginary part. The real part of the dielectric constant, $\varepsilon_{1,r}$, corresponds to the propagation of surface plasma waves and must be negative in value. It is related to the real refractive index, n , and imaginary

refractive index, k , by:

$$\varepsilon_{1,r} = n^2 - k^2 \quad 1.2.2-16$$

The imaginary part of the dielectric constant, $\varepsilon_{1,i}$ governs the damping of the plasmon waves and is related to the real optical constant, n , and imaginary optical constant, k , by:

$$\varepsilon_{1,i} = 2nk \quad 1.2.2-17$$

Surface plasmons are produced when the two wave vectors, K_{sp} and $k_{x,g}$, are equal.

$$\frac{\omega}{c} \sqrt{\frac{\varepsilon_1 \varepsilon_2}{(\varepsilon_1 - \varepsilon_2)}} = \frac{\omega}{c} \sqrt{\varepsilon_0} \sin \theta \quad 1.2.2-18$$

Thus, given dielectric constants of the silver $\varepsilon_{1,r}$, air ε_2 , glass ε_{rg} , and frequency ω , one SPR angle (θ_r) exists. A typical plot for silver is shown in Figure 1.2. The p-polarized second harmonic 532-nm wavelength of a Nd-Hyper YAG laser is used to perform SPR research. The corresponding SPR angle θ_r is calculated to be approximately 43.2°. The choice of metal is usually determined by a sharp dip in its SPR angular profile. The reflectance of light under off-resonance conditions should be high, while a decrease in the reflectance at θ_r should be observed. It is seen that θ_r does not change significantly when using high wavelengths, but it does change significantly at low wavelengths.

Ultimately, two important experiments will be performed with the SPR-LDI/LA-TOFMS instrument. The first experiment is the dependence of mass spectral peak intensity with the angle of incidence. The second experiment is the dependence of mass spectral peak intensity with different silver film thicknesses. Since the above model did

Figure 1.2 Plot of wavelength versus SPR resonance angle for silver.

not take the metal film thickness and the angles of incidence into account, an upgraded approach must be used. To include these parameters into the model, a detailed analysis of the EM wave propagating through each medium must be sought using the Maxwell equations. Predictions about the behavior of the EM wave have already been mentioned. At the SPR angle θ , the thin silver film will absorb the EM wave. However, the degree of attenuation of the EM wave within the silver medium is strongly affected by the thickness d of the silver film. Thick silver films will attenuate the EM wave strongly, while thin silver films will not.

1.2.3 Reflectance Dependence on the Angle of Incidence

During SPR-LDI-TOFMS, the laser fluence changes with the angle of incidence and correlates with reflectance measurements (Lee et al., 1992). New equations must be derived to establish the relationship between the reflectance and the angle of incidence on the backside of the thin silver film. The reflectance dependence on the angles of incidence will be derived for multiple media to enable development of SPR work and SPR-LDI/LA-TOFMS work. Using Eq. 1.2.2-9, Eq. 1.2.2-10, and Eq. 1.2.2-11, the electric field can be described for the incident, reflected and transmitted waves within a non-magnetic medium (Kovacs, 1982):

$$\begin{aligned} \mathbf{E}^{(0)+} &= E_{+}^{(0)+} e^{[ik_{0z}z]} (1, 0, -k_x/k_{0z}) + E_{-}^{(0)+} e^{[-ik_{0z}z]} (1, 0, k_x/k_{0z}) & z \leq z_0 = 0 \\ \mathbf{E}^{(j)+} &= E_{+}^{(j)+} e^{[ik_{jz}(z-z_{j-1})]} (1, 0, -k_x/k_{jz}) + E_{-}^{(j)+} e^{[-ik_{jz}(z-z_{j-1})]} (1, 0, k_x/k_{jz}) & z_{j-1} \leq z \leq z_j \\ \mathbf{E}^{(n)+} &= E_{+}^{(n)+} e^{[ik_{nz}(z-z_{n-1})]} (1, 0, -k_x/k_{nz}) & z_{n-1} \leq z \end{aligned} \quad 1.2.3-1$$

The subscript j denotes the medium of interest. The + subscript represents the electric field wave traveling in the forward z -direction and the – subscript represents the electric field wave traveling in the backward z -direction. Notice that the common factor $\exp[i(k_x x - \omega t)]$ can be omitted because it is constant throughout the media. The bracket term beside each electric field term are the contributions of that portion of the wave in the x , y and z directions. Hence, the first equation describes the initial electric field wave traveling through the first medium, denoted by the superscript 0. It also describes the reflected portion of the electric field wave in the first medium due to the first and second media boundary. The second equation describes the transmitted electric field wave

originating from the previous medium, or the $j-1^{th}$ medium. The transmitted electric field wave travels through the j^{th} medium and reflects a portion of itself at the j^{th} and $j-1^{th}$ medium boundary. The last equation describes the transmitted electric field wave from the $j-1^{th}$ medium traveling through a last medium, the n^{th} medium. Notice that there is no reflected portion of this electric field wave.

The next stage of this endeavor is to determine the magnetic fields in each of the above cases. This is easily accomplished by remembering the relationship:

$$\mathbf{k} \times \mathbf{E} = \mathbf{B} \quad 1.2.3-2$$

This results in the following equations:

$$\begin{aligned} \mathbf{B}^{(0)} &= \frac{\omega}{c} E_z^{(0)} e^{ik_{0z}z} (0, \varepsilon^{(0)} / k_{0z}, 0) + \frac{\omega}{c} E_z^{(0)} e^{-ik_{0z}z} (0, -\varepsilon^{(0)} / k_{0z}, 0) \quad z \leq z_0 = 0 \\ \mathbf{B}^{(j)} &= \frac{\omega}{c} E_z^{(j)} e^{ik_{jz}(z-z_{j-1})} (0, \varepsilon^{(j)} / k_{jz}, 0) + \frac{\omega}{c} E_z^{(j)} e^{-ik_{jz}(z-z_{j-1})} (0, -\varepsilon^{(j)} / k_{jz}, 0) \quad z_{j-1} \leq z \leq z_j \\ \mathbf{B}^{(n)} &= \frac{\omega}{c} E_z^{(n)} e^{ik_{nz}(z-z_{n-1})} (0, \varepsilon^{(n)} / k_{nz}, 0) \quad z_{n-1} \leq z \end{aligned} \quad 1.2.3-3$$

The z-component of the wave vector through the j^{th} medium k_{jz} is given by:

$$k_{jz} = \sqrt{\varepsilon^{(j)} (\omega / c)^2 - k_x^2} \quad 1.2.3-4$$

Eq. 1.2.3-4 is identical to Eq. 1.2.2-10. It is important to realize that the x component of the propagation vector remains constant throughout each medium.

By imposing similar boundary conditions to those stated in Section 1.2.1, the continuity of the tangential (or x-component) of the electric field and the normal (or y-component) of the magnetic field of the p-polarized light wave at each media interface must be met. At the n^{th} interface when $z=z_{n-1}$, the continuity of the electric field component E_x is:

$$E_{-}^{(n-1)} e^{ik_{n-1,z}d_{n-1}} + E_{-}^{(n-1)} = E_{-}^{(n)} \quad 1.2.3-5$$

Similarly, the continuity of the magnetic field component B_y is:

$$E_{-}^{(n-1)} e^{ik_{n-1,z}d_{n-1}} (\varepsilon^{(n-1)} / k_{n-1,z}) + E_{-}^{(n-1)} (-\varepsilon^{(n-1)} / k_{n-1,z}) = E_{-}^{(n)} (\varepsilon^{(n)} / k_{nz}) \quad 1.2.3-6$$

The term d_{n-1} is the thickness of the $(n-1)^{th}$ medium. Using simple algebra, $E_{-}^{(n)}$ and $E_{-}^{(n-1)}$ can be determined using Eq. 1.2.3-5 and Eq. 1.2.3-6. As previously mentioned, the subscripts on each term denote the direction of the p-polarized light wave. Hence, the first of these two terms is the transmitted portion of the p-polarized light wave, while the second of these terms is the reflected portion of the p-polarized light wave. Since the reflected portion in the $(n-1)^{th}$ medium of the p-polarized light wave is desired, $E_{-}^{(n-1)}$ is solved:

$$E_{-}^{(n-1)} = r_{n-1,n} e^{ik_{n-1,z}d_{n-1}} E_{-}^{(n)} \quad 1.2.3-7$$

The term $r_{n-1,n}$ is the reflected portion of the p-polarized light wave between the $(n-1)^{th}$ and the n^{th} media:

$$r_{n-1,n} = \frac{X_{n-1} - X_n}{X_{n-1} + X_n} \quad 1.2.3-8$$

Lastly, the terms X_{n-1} and X_n describe the p-polarized light wave through the $(n-1)^{th}$ medium and are written for any j^{th} medium as:

$$X_j = \varepsilon^{(j)} / k_{jz} \quad 1.2.3-9$$

By performing this procedure recursively, many equations can be derived to describe the reflected portion of the p-polarized light wave through the $(j-1)^{th}$ and j^{th} media. However, once the equation for the initial medium is derived (i.e. $j=0$), the procedure terminates. A general equation for the reflected portion of the p-polarized light

wave between the 0^{th} and the n^{th} media can be written:

$$E_-^{(0)} = E_-^{(0)} r_{0n} \quad 1.2.3-10$$

In this case:

$$r_{0n} = \frac{r_{01} + r_{1n} e^{2ik_{1z}d_1}}{1 + r_{01}r_{1n} e^{2ik_{1z}d_1}} \quad 1.2.3-11$$

The reflected portion of the p-polarized wave through media that are adjacent to each other use Eq. 1.2.3-8.

By setting the initial electric field in medium 0 to unity, the reflectance or the reflected light intensity R can be written as:

$$R = |E_-^{(0)}|^2 / |E_-^{(0)}|^2 = |E_-^{(0)}|^2 \quad 1.2.3-12$$

The reflectance can be determined if the angle of incidence of the p-polarized light wave in medium 0, the dielectric constants of all the media, the thickness of each medium, and the wavelength of the incident light is known. The plot of the reflectance against the angles of incidence is termed the SPR angular profile. The plot illustrates a typical dip in the reflectance at the SPR angle. The imaginary term of the dielectric constant of the metal (primarily a function of the metal's conductivity) determines the width of the SPR dip. Appendix 3.2 states a Fortran program that was written to perform these calculations. The corresponding SPR angular profiles for different thickness of a thin silver film using the 532-nm wavelength are shown in Figure 1.3. The variation in the reflectance at the SPR angle θ_r at the different silver film thickness is illustrated in

Figure 1.3 Plot of reflectance against angle of incidence using a 532-nm wavelength for different thin silver film thicknesses.

Figure 1.4 Variation in reflectance at the SPR angle θ_r for different silver film thicknesses.

Figure 1.4.

However, a realization and limitation about this derivation is that all of the media are considered to be homogeneous and uniform in thickness and surface morphology. In our experiment, however, only the glass (medium 0) and silver (medium 1) are uniform in thickness and surface morphology. If air is used as the second medium, the derivation still applies. Unfortunately, if a sample is deposited on the surface of the silver film, the derivation does not apply because the sample is heterogeneous and non-uniform on the scale of the laser spot size. This condition must be emphasized because it is the latter case (i.e. a sample medium) that will be used to perform SPR-LDI/LA-TOFMS experiments.

1.2.4 Heterogeneous Modeling

During sample preparation for mass spectrometric analysis, chemical reagents are often added to enhance the analytical sensitivity. Consequently, the modeling work in Section 1.2.3 must be altered to include an equivalent dielectric constant for the sample medium. As an electric field is induced in a sample medium, an appropriate capacitance can be associated with it. Since the medium is heterogeneous, n capacitors in series and parallel can be set up to produce an equivalent dielectric constant ϵ_r of the entire sample. Given the dielectric constants $\epsilon_{r,i}$ and the volume ratios V_i of the individual components within the sample medium:

$$\epsilon_r^k = \sum_{i=1}^n V_i \epsilon_{r,i}^k \quad 1.2.4-1$$

The term k can vary between -1 and 1. These limits are known as the Weiner limits.

Previous studies involving heterogeneous media showed that the dielectric constant of the entire medium was closely predicted by Eq. 1.2.4-1, with k ranging from 0 to 1/2. It is important to realize that according to this model, if the difference between the dielectric constants of two components is great, there will be a larger range of an equivalent dielectric constant that the entire medium may have.

The two components involved in this work are a drug (representing a relatively small organic analyte) and a salt. The salt can be the same metal as the SPR metal film, silver in this work, and ideally it should have a dielectric constant close to the drug to reduce the range of equivalent dielectric constants ($\epsilon_{r,drug} \approx 20$). A typical profile can be

reduce the range of equivalent dielectric constants ($\epsilon_{r,drug} \approx 20$). A typical profile can be seen in Figure 1.5 for AgCl, which has a dielectric constant of 11.2.

Figure 1.5 Plot of dielectric constant ϵ_r versus volume ratio V .

1.2.5 Techniques to Monitor SPR Activity

The SPR characterization apparatus can be seen in Figure 1.6. Red light, emitted from a light emitting diode (LED), travels through a p-polarizer and is focused through a glass prism onto the backside of the thin silver film. Since the thin silver film is coupled with oil to the glass prism, the thin silver film absorbs the light at θ_r . The intensity of the reflected light is then imaged onto a photodiode array (PDA) detector. The intensity of the reflected light (or the reflectance) versus the pixel number of the array (which can be calibrated into an angle of incidence) is displayed as an SPR angular profile on a computer. This is illustrated in Figure 1.7 A.

Alternatively, a single light pulse can travel through a polarizer and glass prism to strike the backside of a thin silver film (coupled with oil to the glass prism) at one angle of incidence. The thin silver film will absorb the light at θ_r greater than other angles of incidence. Since SPR is a light absorption process followed by non-radiative relaxation, heating of the thin silver film will occur. The resulting volume change can be measured by means of a piezoelectric transducer coupled to a digital storage oscilloscope (DSO). This technique is called photoacoustic spectroscopy (PAS). Since the thin silver film will absorb the light most intensely at θ_r , the photoacoustic signal will be largest at this angle of incidence. By changing the angle of incidence that the light strikes the thin silver film, a SPR angular profile can be obtained. This is illustrated in Figure 1.7 B. Clearly, the resolution achieved with the PDA detector is better than that with

Figure 1.6 SPR characterization apparatus.

Figure 1.7 SPR angular profile of a thin silver film obtained using: (A) a photodiode array, and (B) a piezoelectric transducer.

the PAS technique using a focused laser beam. although attaching a piezoelectric transducer greatly simplifies the instrumentation.

1.3 Time-of-Flight Mass Spectrometry (TOFMS)

1.3.1 Pressure and Collision Probability

A typical mass spectrometer must have base pressures on the order of 10^{-6} to 10^{-7} torr. Attaining a low base pressure will contribute to the low collision probability of an ion with other neutral and ionic species within the gas-phase. With a low base pressure and hence low collision probability, detection of an ion is greatly facilitated because the mean free path L is larger. The effect of the base pressure on the mean free path L is described below.

Actual molecules have a finite size. In the derivation given by Wehr et al., these molecules are assumed to be rigid spheres (Wehr et al., 1985). Thus, at the instant of a collision between two molecules, one target molecule with radius r_t comes into contact with one bullet molecule with radius r_b . When the collision occurs, the distance between the centers of these molecules is the sum of their radii, $r_t + r_b$.

Consider a thin layer of material with dimensions l , l , and dx with target molecules at rest. If N bullet molecules strike the plane, some bullet molecules will strike the target while others will not. The ratio of the number of collisions dN to the total number of bullets is given by:

$$\frac{dN}{N} = \frac{area_{target}}{area_{total}} \quad 1.3.1-1$$

The target area σ of a single molecule, defined as the sum of the target molecule area and the bullet molecule area is written as:

$$\sigma = \pi(r_t + r_b)^2 \quad 1.3.1-2$$

If there are n target molecules per unit volume, the total target area is determined to be:

$$n\sigma l^2 dx \quad 1.3.1-3$$

By substituting Eq. 1.3.1-3 and knowing that the total area of the layer is l^2 , Eq. 1.3.1-1 can be rewritten as:

$$\frac{dN}{N} = \frac{n\sigma l^2 dx}{l^2} = n\sigma dx \quad 1.3.1-4$$

The ratio dN/N is defined as the probability of collision. Thus, the probability of a collision between two molecules is directly proportional to the area σ of a single molecule.

If there are N_0 bullet molecules per unit area that are on the face of a layer of a material of finite thickness dx , then the number of bullet molecules N that travel through the layer can be determined by integrating Eq. 1.3.1-4:

$$\int_{N_0}^N -\frac{dN}{N} = \int_0^x n\sigma dx \quad 1.3.1-5$$

This results in the exponential equation:

$$N = N_0 e^{-n\sigma x} \quad 1.3.1-6$$

The average distance that a bullet molecule travels between collisions is defined as the mean free path L . The mean free path L is regarded as the ratio between the total distance traveled by N molecules and the total number of collisions that have occurred.

From Eq. 1.3.1-5, N bullet molecules traverse a thickness dx . During their flight, dN number of collisions occur. The total distance traveled by all of the N bullet molecules is Ndx . Hence, the mean free path L is given by:

$$L = \frac{Ndx}{Nn\sigma dx} = \frac{1}{n\sigma} \quad 1.3.1-7$$

In this derivation, the target molecules are assumed to be at rest. In the mass spectrometer, however, target and bullet molecules move randomly. Head-on collisions occur, but molecules can also sideswipe each other, which introduces a y or z-component of the distance. Since there are two components that determine the sideswipe, the total distance traveled by the N bullet molecules is given by:

$$distance_{total} = \sqrt{2}Ndx \quad 1.3.1-8$$

An additional 1/2 must be multiplied to the mean free path L so that each collision is not counted twice, once as the bullet molecule colliding with the target molecule and once as the target molecule colliding with the bullet molecule. The final equation that describes the mean free path L is written below:

$$L = \frac{\sqrt{2}}{2n\sigma} \quad 1.3.1-9$$

Clearly, the mean free path decreases as n increases.

If the molecules behave as an ideal gas, the n target molecules per unit volume are related to the pressure P , the temperature T , the Avogadro constant N , and the gas constant R :

$$n = \frac{PN}{RT} \quad 1.3.1-10$$

The mean free path L is related to the pressure P inside the mass spectrometer by:

$$L = \frac{RT\sqrt{2}}{2NP\sigma}$$

1.3.1-11

In a mass spectrometer, the temperature is constant. Given this condition, the lower the pressure P , the longer the mean free path L . Figure 1.8 illustrates the mean free path length L as a function of the pressure P . At a low pressure P , the mean free path L of the bullet molecule increases drastically with a slight decrease in P , while at a higher pressure P , the mean free path L of the bullet molecule increases a little. Lastly, if the pressure P decreases by one order of magnitude, then the mean free path L will increase by one order of magnitude. To achieve a low pressure, a high vacuum pump like a turbo molecular pump or a diffusion pump is required. In our laboratory, a diffusion pump is used to evacuate a time-of-flight mass spectrometer (TOFMS) to reach a base pressure of 10^{-6} to 10^{-7} torr, as shown in Figure 1.9.

Diffusion pumps work on the principle of momentum transfer, where heavy oil vapor molecules collide with gas molecules to ultimately exhaust them to the atmosphere. Vapor molecules are produced at the base of the diffusion pump by heating a suitable pumping fluid (Varian Neovac SY Diffusion Pump Fluid). The chemical and physical properties of the pumping fluid are heavy, non-reactive and have a very low vapor pressure. The fluid vapor rises up the middle column of the diffusion pump and strikes a downward-directed jet assembly. The vapor forms a molecular curtain and collides with a side of the diffusion pump. The side of the diffusion pump is cold because cold water is circulated on the outside. Then, the vapor condenses and returns to the diffusion pump base to be reheated. In effect, the vapor maintains a steady reflux action. As the vapor refluxes, gas molecules from the mass spectrometer enter the middle column to collide

Figure 1.8 Plot of mean free path L versus pressure P .

Figure 1.9 Pumping curve of the Edwards 'Speedivac' E04 Vapor Diffusion Pump.

with the vapor. Once the vapor is at the base of the diffusion pump, the gas molecules are pumped out by a roughing pump. Hence, a roughing pump or rotary pump is required for backing the diffusion pump.

Another function of the roughing pump is to evacuate the mass spectrometer before the diffusion pump evacuates the mass spectrometer. In this way, the cost of maintaining the mass spectrometer is reduced because the diffusion pump oil does not require frequent replacement. Also, potentially reactive chemicals that may damage the diffusion pump, will damage the roughing pump instead, which is less expensive than the diffusion pump.

1.3.2 Flight Time of an Ion

Since large-molecular-weight compounds will be analyzed, a TOFMS with good resolution, simple construction, easy manipulation and modifications was desired. Furthermore, the SPR-LDI/LA process should be easily incorporated. Unfortunately, the primary disadvantage of a TOFMS is the limited resolution. The design chosen for this TOFMS was a double-focusing field system due to its optimal resolution (Wiley and McLaren, 1955). The construction of a double-focusing field system is illustrated in Figure 1.10. The total time T for an ion with mass m and charge q to travel through a TOFMS is the sum of the separate times it takes to travel through the different stages of the spectrometer. The double-focusing field system has two acceleration regions and a drifting region. Hence, the total flight time T is the sum of the flight time in the first acceleration region T_s , the flight time in the second acceleration region T_d , and the flight time in the field-free drift tube T_D :

$$T = T_s + T_d + T_D \quad 1.3.2-1$$

In the acceleration region, the ion experiences the force F_x from the electric field E_x along the x (or axial) direction, and will accelerate along the x-direction with acceleration a_x . If the initial energy of the ion is assumed to be 0, and therefore the initial velocity of the ion is 0, then Newton's second law of motion for the ion is described by:

$$F_x = ma_x = qE_x \quad 1.3.2-2$$

Notice that only the x-component of the force needs to be considered because the electric field is assumed to be across two infinitely-long parallel planes. From classical

Figure 1.10 The Wiley and McLaren double-focusing TOFMS system with optimal resolution.

mechanics, the distance traveled by the ion x is related to the ion's acceleration a_x and the ion's traveling time t :

$$x = \frac{1}{2}a_x t^2 \quad 1.3.2-3$$

Rearranging Eq. 1.3.2-3 to solve for the acceleration, and substituting this equation into Eq. 1.3.2-2 results in:

$$m\left(\frac{2x}{t^2}\right) = qE_x \quad 1.3.2-4$$

Thus the time for an ion to spend in the acceleration region is:

$$t = \frac{(2m)^{\frac{1}{2}} \sqrt{qx E_x}}{qE_x} \quad 1.3.2-5$$

Since there are two acceleration regions, the total flight time equation will contain two time components, each given by Eq. 1.3.2-5. In the first time component, which corresponds to the first acceleration region in Figure 1.10, the distance x is replaced by the distance s and the time t is replaced by the time T_s . The first equation is rewritten as:

$$T_s = \frac{(2m)^{\frac{1}{2}} \sqrt{qs E_s}}{qE_s} \quad 1.3.2-6$$

In the second time component, which corresponds to the second acceleration region in Figure 1.10, the distance x is replaced by the distance d , the energy term qdE_d is replaced by the difference between the final and initial energies of the ion $[(qdE_d + qsE_s)^{1/2} - (qsE_s)^{1/2}]$, and the time t is replaced by the time T_d . This equation is slightly more complicated because the time T_d is quadratic. The second equation is rewritten as:

$$T_d = \frac{(2m)^{\frac{1}{2}} \sqrt{(qdE_d + qsE_s)^{\frac{1}{2}} - (qsE_s)^{\frac{1}{2}}}}{qE_d} \quad 1.3.2-7$$

The time t of the ion traveling at velocity v spends in the drift tube of distance x is described by classical mechanics:

$$x = vt \quad 1.3.2-8$$

Since the energy of the ion in the drift region does not change, the energy of the ion accumulated by the acceleration regions will be its kinetic energy:

$$KE = \frac{1}{2}mv^2 = qdE_d + qsE_s, \quad 1.3.2-9$$

Rearranging Eq. 1.3.2-9 to solve for the velocity v and substituting into Eq. 1.3.2-8 gives:

$$x = \left(\frac{(2qdE_d + 2qsE_s)}{m} \right)^{\frac{1}{2}} t \quad 1.3.2-10$$

Rearranging Eq. 1.3.2-10 to solve for the time t taken for the ion to travel the distance x :

$$t = (2m)^{\frac{1}{2}} \frac{x}{2\sqrt{qdE_d + qsE_s}} \quad 1.3.2-11$$

When the ion arrives at the ion collector, the time T_D replaces time t and the distance D replaces the distance x . The time for the ion to drift the length of the field-free drift tube is:

$$T_D = (2m)^{\frac{1}{2}} \frac{D}{2\sqrt{qdE_d + qsE_s}} \quad 1.3.2-12$$

Substitution of T_s , T_d and T_D , into Eq. 1.3.2-1, and expressing the total energy of the ion as U_i , gives the total flight time of the ion:

$$T = 1.02 \left(\frac{m}{2U_t} \right)^{\frac{1}{2}} \left(2k_0^{1/2} s + \frac{2k_0^{1/2}}{k_0^{1/2} + 1} d + D \right) \quad 1.3.2-13$$

In this equation, the total energy of the ion U_t is:

$$U_t = qsE_s + qdE_d \quad 1.3.2-14$$

and the term k_0 is given by:

$$k_0 = \frac{(sE_s + dE_d)}{sE_s} \quad 1.3.2-15$$

From Eq. 1.3.2-13, the flight time of the ion is directly proportional to the square root of its mass.

1.3.3 Space Resolution

The total flight time of an ion traveling through two acceleration fields and one drift tube has been derived in Eq. 1.3.2-13. The difference in flight time between adjacent masses must be maximized. This is accomplished by setting $dT/ds=0$, described below. If the distances D , s and d are known, k_0 and hence E_d/E_s can be determined.

Assuming that the internal energy of the ions is 0, the drift tube length D is derived:

$$D = 2sk_0^2 \left[1 - \frac{l}{k_0 - k_0^2} \left(\frac{d}{s} \right) \right] \quad 1.3.3-1$$

At a distance s_0 within the first acceleration region, the condition of $dT/ds=0$ will have either a maximum, a minimum or a point of inflection. At the point of inflection, $d^2T/ds^2=0$, another focusing condition is derived:

$$\frac{d}{s} = \left(\frac{k_0 - 3}{k_0} \right) \frac{D}{2s} \quad 1.3.3-2$$

Assuming the internal energy of the ion is 0, the time $T(s_0)$ is a minimum when the left-hand side of Eq. 1.3.3-2 is larger than the right-hand side. When $T(s_0)$ is a maximum, the left-hand side of Eq. 1.3.3-2 is smaller than the right-hand side. For a maximum $T(s_0)$, k_0 must also be larger than 3.

The change in total flight time ΔT_m of an ion corresponding to a small change in position Δs about a point s_0 within the first acceleration region can be written as a Taylor expansion:

$$\Delta T_{\Delta s} = \sum_{n=1}^{\infty} \frac{1}{n!} \left(\frac{d^n T}{ds^n}(s) \right)_{s_0} (\Delta s)^n \quad 1.3.3-3$$

To measure the resolution of the separated masses, the time difference between adjacent masses is calculated from Eq. 1.3.2-13:

$$T_{m+1} - T_m = \left[\left(1 + \frac{1}{m} \right)^{\frac{1}{2}} - 1 \right] T_m \approx \frac{T_m}{2m} \quad 1.3.3-4$$

From the first focusing condition ($n=1$), the first term of Eq. 1.3.3-3 is zero. However, the second focusing condition is non-zero if $T(s_0)$ is a maximum. The second term in Eq. 1.3.3-3 is evaluated by replacing Δs with the maximum deviation of s from s_0 , $\Delta s/2$. The change in total flight time ΔT_m of an ion is equated to the difference between two adjacent masses (the right hand side of Eq. 1.3.3-4) to give the space resolution M_s :

$$M_s \approx 16k_0 (s_0 / \Delta s)^2 \quad 1.3.3-5$$

The space resolution is good for s_0 larger than Δs . Since D/s at $s=s_0$ determines the value of k_0 from Eq. 1.3.3-1, D should be large. Increasing d also improves the space resolution.

1.3.4 Energy Resolution

Another important aspect to consider when building a TOFMS instrument is the energy resolution. Energy resolution is defined as a time spread due to the kinetic energy distribution of ions with a given mass. Clearly the kinetic energy distribution must be small in order to optimize the energy resolution. This is accomplished by considering the time spread between two ions with identical mass that are formed in the first acceleration region at the same initial position s . Both of these ions have an internal energy U_0 , but they have velocities that are opposite to each other, as shown in Figure 1.10. One of the ions is moving towards the ion collector, while the other ion is moving toward the source repeller plate. This latter ion will be decelerated by the electric field E_s until it stops. It will then be accelerated towards the ion collector, and return to its initial position s with its original velocity v_0 . The time taken for the above deceleration and acceleration is called the turn-around time. This causes a time spread ΔT_θ which is twice the deceleration time. Since the ions have an internal energy U_0 , the time spread is given by:

$$\Delta T_\theta = 1.02 \frac{2v_0 m}{qE_s} = 1.02 \frac{2(2m)^2 \sqrt{U_0}}{qE_s} \quad 1.3.4-1$$

This equation has the same form as Eq. 1.3.2-6 with the energy term $qsEs$ substituted by the internal energy U_0 of the ion.

Taking the energy distribution of the ions only into consideration, the maximum resolvable mass, M_θ (or the energy resolution) is the mass between two adjacent masses, given by Eq. 1.3.3-4:

$$M_\theta = \frac{T}{2\Delta T_\theta} \quad 1.3.4-2$$

Assuming the ions have an internal energy U_θ , substitution of Eq. 1.3.2-13 and Eq. 1.3.4-1 into Eq. 1.3.4-2, and using the distances calculated from Eq. 1.3.3-1 (the first focusing condition), the energy resolution M_θ is written as:

$$M_\theta = \frac{1}{4} \left(\frac{U_i}{U_o} \right)^2 \left(\frac{k_o + 1}{k_o^2} - \frac{k_o^2 - 1}{k_o + k_c^2} \left(\frac{d}{s_o} \right) \right) \quad 1.3.4-4$$

By increasing D and/or decreasing s_o and d , M_θ increases.

1.3.5 Total Resolution

To determine the total resolution M of the double-field focusing system, the space resolution and the energy resolution must be considered. Upper and lower bounds of the total resolution are intuitively obvious. The total resolution must be smaller than the space resolution M_s and/or the energy resolution M_θ . Also, the total resolution M will be at least as large as the overall resolution $M_{s,\theta}$ assuming the total time spread to be the sum of the energy and space time spreads:

$$\frac{1}{M_{s,\theta}} = \frac{1}{M_s} + \frac{1}{M_\theta} \quad 1.3.5-1$$

If the lengths and voltages to guide the ion to the ion collector are known, $M_{s,\theta}$ can be calculated. The small change in position Δs is given a value of 0.2 cm, and the internal energy of the ions (primarily thermal energy) U_0 is given a value of 0.09 eV. Thus, the overall resolution $M_{s,\theta}$ of our TOFMS could be calculated, as described in Section 1.3.6.

1.3.6 Construction of the SPR-LDI/LA-TOFMS

To determine the placement of the grids, the voltages on the grids (that provide uniform electric fields) and the distances of the three regions for the SPR-LDI/LA-TOFMS instrument, the total length of the SPR-LDI/LA-TOFMS must be known first. Using the double-focusing field system of Wiley and McLaren as a test, the total length of their TOFMS instrument was 41.4 cm. An EXCEL program was written to determine the values of s and d given the drift tube length D and the voltages applied to grids a, b and c. This program was applied to the Wiley and McLaren TOFMS instrument. The drift tube length D (40 cm), the total length $s + d$ (1.4 cm), the voltages at grids a (1600 V), b (1536 V) and c (0 V), and an arbitrary mass m (50 Da) were assigned. Two columns of length s and length d were created to sum to the total length of 1.4 cm. The electric fields E_s and E_d were calculated, and the theoretical flight times for masses 50 and 51 were determined using Eq. 1.3.2-13, Eq. 1.3.2-14 and Eq. 1.3.2-15. To optimize the space focusing, the right-hand side of Eq. 1.3.3-1 (for the drift tube length D) was calculated. This value was then compared to the actual drift tube length D (40 cm). When both of these values were equal, the value of s and d were determined to be 0.2 cm and 1.2 cm respectively for optimum space resolution.

Given the voltages of grids a, b and c and the length $s + d$, the relationship between the optimum lengths for s and d with the drift tube length D is illustrated in Figure 1.11 A. These relationships are linear. For the Wiley and McLaren values, the equation that determines the optimum length d is $d = -0.0039D + 1.3548$, while the

Figure 1.11 Relationship between optimum lengths for s and d with drift tube length D . (A) The length $s + d = 1.4$ cm, grid $a = 1600$ V, grid $b = 1536$ V, (B) The length $s + d = 2.86$ cm, grid $a = 1600$ V, grid $b = 1536$ V, and (C) The length $s + d = 2.86$ cm, grid $a = 3000$ V, grid $b = 2880$ V.

equation that determines the optimum length s is $s=0.0039D + 0.0452$. When $D=40$ cm, d was determined to be 1.2 cm, and s was determined to be 0.2 cm.

The total length between our ion collector, or a micro-channel plate (MCP) detector of the SPR-LDI/LA-TOFMS and the location of the thin silver film was determined to be 51.47 cm. Since the MCP was operating at a front-face voltage of -1.6 kV, a small distance of approximately 6.41 cm was kept between the end of the drift tube (or grid D) and the MCP detector. A drift tube length of 42.2 cm was chosen arbitrarily. Thus, the length $s + d$ was calculated to be 2.86 cm. When this length $s + d$ was plugged into the EXCEL program, a similar relationship between the optimum lengths for s and d with the drift tube length D was observed, as illustrated in Figure 1.11 B. The linear equation that determines the optimum length d is $d=-0.0039D + 2.7677$, while the equation that determines the optimum length s is $s=0.0039D + 0.0923$. The only parameter in the EXCEL program that was altered was the length $s + d$, and this resulted in the new y-intercepts. Using these linear equations for the drift tube length D of 42.2 cm, the optimum length d was determined to be 2.60 cm, and the optimum length s was determined to be 0.26 cm. The sum of these two values was 2.86 cm, confirming that this EXCEL program worked.

To introduce flexibility into the SPR-LDI/LA-TOFMS instrument, the same EXCEL program was used for different voltages applied to grids a, b and c. Since the maximum voltage rating of a MHV feedthrough is approximately 3500 kV, the grid a voltage was changed to 3000 V and the grid b was changed to 2880 V. The voltages were chosen to keep the k_0 value the same according to Eq. 1.3.2-15. The same length $s + d$ was kept as above. The results are illustrated in Figure 1.11 C. The equation of the

line was the same. At $k_0=25$ and $U_i=3000$ V, the time spread ΔT_θ at different s and d values was calculated to determine the difference between high and low E_s and E_d settings. The difference in the flight time T_m between masses 50 and 51 was also calculated. The time spread ΔT_θ plotted against $T_{m-1}-T_m$ for an internal energy of 0.09 eV is illustrated in Figure 1.12. However, Figure 1.12 A (grid a is 1600 V and grid b is 1536 V) indicates a linear dependence described by the equation $y=0.2827x + 0.0068$. When the higher voltages are used (grid a is 3000 V and grid b is 2880 V), the linear equation becomes $y=0.2064x + 0.0037$. The time spread ΔT_θ is small using higher voltages. Using Eq. 1.3.3-5, Eq. 1.3.4-4 and Eq. 1.3.5-1, the resolutions of the SPR-LDI/LA-TOFMS with the low and high voltages are shown in Table 1.1, assuming that the small change in position Δs about a point s_0 is 0.2 cm. The lengths d and s must be measured prior to sealing the vacuum chamber of the SPR-LDI/LA-TOFMS instrument.

Table 1.1 Table of space resolution, M_s , the energy resolution, M_θ and the overall resolution $M_{s,\theta}$ for different voltages.

	Space resolution, M_s	Energy resolution, M_θ	Overall resolution, $M_{s,\theta}$
Va=1600, Vb=1536	676	128.9	108.3
Va=3000, Vb=2880	676	176.5	140.0

Figure 1.12 Relationship between time spread ΔT_θ and difference in flight time $T_{m+1} - T_m$ for an ion of mass 50 with an internal energy of 0.09 eV. The length $s + d$ is 2.86 cm. (A) grid a=1600 V, grid b=1536 V, and (B) grid a=3000 V, grid b=2880 V.

Chapter 2: Development of the (SPR)-LDI/LA-TOFMS Instrument

2.1 First Configuration

2.1.1 Description

With the existing holes on the vacuum chamber top, the first configuration of the LDI/LA-TOFMS instrument was oriented vertically, as shown in Figure 2.1. The desorption/ablation of either a Lumonics thin silver film or a sample deposited on its surface generated ions that were detected with the LDI/LA-TOFMS instrument. Either a 532-nm wavelength (second harmonic) or 355-nm wavelength (third harmonic) output beam from a Nd-Hyper YAG laser was employed. The repetition rate of the laser pulses was selected with the local control unit (LCU). A right-angle prism outside the LDI/LA-TOFMS directed the laser beam into the ion source chamber. The laser beam was focused with a lens, passed through a quartz window port and directed again with a right-angle prism mounted inside the ion source chamber. Finally, the laser beam formed to a spot size of 0.1 mm^2 on the backside of a Lumonics thin silver film with or without a deposited sample. The thin silver film was coupled to the right-angle prism with diffusion pump oil (Varian Neovac SY).

Some of the electrical connections for the LDI/LA-TOFMS instrument were manufactured while others were not. A BNC high-voltage cable from a Fluke high voltage power supply (model 4088) was connected to a homemade grid controller. The voltage divider within the homemade grid controller provided three output voltages A, B and C. These potentials were fed with three BNC cables to three MHV feedthroughs.

Figure 2.1 A Top view of the LDI/LA-TOFMS instrument in the first configuration. The aluminum sample holder is not shown.

Figure 2.1 B Side view of the LDI/LA-TOFMS instrument in the first configuration.

Figure 2-1 C Another side view of the LD/LA-TOFMS instrument in the first configuration.

These feedthroughs were electrical connections that each transferred a potential from the outside to the inside of the LDI/LA-TOFMS without breaking the high vacuum seal. Voltage C was connected to a MHV feedthrough on the MCP detector. Voltage A was connected to a SHV feedthrough and voltage B was connected to the MHV feedthrough on the ion source chamber. Inside the ion source chamber, a wire connected the SHV feedthrough to an aluminum holder. A positive voltage of ~ 3 kV was applied to the aluminum holder. With copper fingers, the aluminum holder was connected to the Lumonics thin silver film. Now the thin silver film was also acting as a repeller plate and grid A. Inside the ion source chamber, a different wire connected the MHV feedthrough to grid B, which was mounted on an adjustable tube. A slightly lower positive potential of ~ 2.9 kV was applied to grid B. A third wire connected the voltage C MHV feedthrough on the MCP detector to grid C, which was mounted on an adjustable field-free drift tube. A positive potential of ~ 5 V was applied to grid C to slightly focus the generated ions. A fourth wire connected another MHV feedthrough on the MCP detector to grid D, which was mounted on the other end of the field-free drift tube. This MHV feedthrough and hence grid D was grounded to the LDI/LA-TOFMS instrument. A homemade power supply was connected to the third MHV feedthrough to operate the MCP detector at a front-face voltage of -1.6 kV. To complete the MCP detector circuit, a fourth MHV feedthrough was grounded to the LDI/LA-TOFMS instrument. Arriving ions were detected with two 25-mm diameter multichannel plates on the MCP detector. As the ions collided with the plates, an electrical signal was generated. Since the multichannels were oriented in a chevron configuration, the electrical signal was amplified by a factor of 5×10^4 . The electrical signal was fed from a fifth MHV

feedthrough on the MCP detector to channel 2 of a 300 MHz LeCroy 9312M Digital Storage Oscilloscope (DSO). This oscilloscope was triggered by an electrical signal fed from the Pockel cell output of the Nd-Hyper YAG laser to channel 1.

The positive voltage on the thin silver film (grid A), the lower voltage on grid B and the even lower voltage on grid C provided two uniform electrical fields. These fields acted on the LDI/LA ions to accelerate them towards the MCP detector. After the ions traveled through grid C, they were separated through differential flight speeds/velocities to a field-free drift tube that was capped at both ends with a grounded grid C and a grounded grid D. After the ions passed through grid D, they were slightly post-accelerated towards the MCP detector with a -1.6 kV potential on the front face of the MCP detector. Ions striking the MCP detector had a total energy equal to the sum of all the energies that were acquired during their accelerations in the several uniform electric fields.

SPR-LDI/LA-TOFMS could be performed in this configuration, as illustrated in Figure 2.2. A p-polarized 532-nm wavelength laser beam traveled through a focusing lens, passed through a quartz window port of the ion source chamber, and fell onto a rotatable mirror. A Hurst Model SAS 4006-006 stepping motor performed mirror rotation at 0.0125 degrees/step, and its movement was calibrated to determine the angle of laser beam incidence. One of these angles would be, of course, the SPR angle θ_r . The p-polarized 532-nm laser beam traveled through a right-angle glass prism (or a semi-cylindrical plexiglas prism) to strike the backside of the thin silver film with or without a sample deposited on its top surface. The thin silver film was coupled to the prism with diffusion pump oil (Varian Neovac SY). The electrical connections were identical to

Figure 2.2 Side view of the SPR-LDI/LA-TOFMS instrument in the first configuration.

those for the LDI/LA-TOFMS instrument.

The mass spectra displayed on the 300 MHz DSO (channel 2) were either sum-averaged or single-shot. They were either stored as digital data for computer spectral analysis, or printed as a hard copy. For computer analysis, proper conversion of the digital data to an ASCII file was performed using a program written in Fortran 77 (see Appendix 3.3). This file was then imported into an EXCEL spreadsheet for mass spectral analysis. Since the square of the flight time of an ion is directly proportional to its mass (see Eq. 1.3.2-13), conversion of the time base to a mass scale was accomplished using an empirical equation $m = kt^2 - b$. The two terms, k and b are the best-fit calibration constant and the mass at zero time, respectively. For a hard copy of the mass spectra, a Hewlett Packard Model 7470A plotter was connected to the DSO.

2.1.2 (SPR)-LDI/LA-TOFMS Preliminary Tests

Preliminary experiments quickly demonstrated several limitations of this first LDI/LA-TOFMS configuration. One of these is a relatively small mass range even though TOFMS is theoretically supposed to have an unlimited mass range. Proteins, whose masses ranged from 12,384 Da (horse heart cytochrome C) to approximately 60,000 Da (bovine serum albumin) were not detectable using this TOFMS. However, gramicidin S, a cyclic decapeptide that has a molecular mass of 1,141 Da, was detected, although a concentration of 4.0 mg/ml or higher was required. MALDI experiments using a mixture of gramicidin S and 2,5-dihydroxybenzoic acid were performed (using a 355-nm wavelength) to improve detection sensitivity. The peak signal intensity exhibited no change. Multiple-spot sampling must be performed to obtain a good analyte signal because a decrease in the signal intensities was observed upon continuous LDI/LA at the same spot. This phenomenon is similar to MALDI experiments. It is well known that a good ionization efficiency is critical in producing a strong signal in a LDI/LA-TOFMS mass spectrum. These preliminary results indicated that the ionization efficiency of our LDI/LA technique was not very good for large molecular masses.

Resolution of this first TOFMS configuration was affected by the present LDI/LA method. A mass spectrum obtained with a low laser fluence was better resolved than one obtained using a high laser fluence. However, a small laser fluence would produce a low peak signal intensity. It seemed a balance in laser fluence must be achieved for good mass spectral resolution at a high precision. Better resolution was also observed when

the silver film voltage was increased from 2115 V to 4500 V. This was expected, as described in the Section 1.3.6.

The effect of the vacuum on our LDI/LA process was also investigated using the first configuration. It is well known that if the vacuum decreases, the mean free path of an ion decreases significantly. Consequently, the number of ions striking the detector decreases and a small peak signal intensity will be observed. As shown in Figure 2.3, upon LDI/LA at one location on the silver film, a small increase in the pressure of the vacuum system was observed for the first two pulses. Heating of the thin silver film was thought to occur, during which a small amount of the silver desorbed. The third pulse ablated the thin silver film. Subsequent laser pulses produced small increases in the pressure. These results suggest that there were microscopic desorption processes occurring during the first two laser pulses, followed by mesoscopic desorption processes during the third pulse. This probably affected the migration of the ions that are generated during this LDI/LA process because the mean free path was decreased. Upon subsequent pulses, the vacuum slowly returned to normal, suggesting that most of the thin silver film was already ablated. Since there are fewer ions in the gas phase, mass spectral peak intensities are expected to decrease. Even though the mean free path of the ions was decreased substantially, multiple-spot sampling must be performed to obtain good mass spectral signal-to-noise in the final sum-averaged LDI/LA-TOFMS spectrum.

For SPR-LDI/LA experiments, a computer program was used to generate a ASCII file for each mass spectrum. A contour plot was accomplished by selecting sections of the ASCII files to create a new ASCII file with three columns. These represented the row number (which was equivalent to the time of flight), an expanded angle parameter (which

Figure 2.3 Effect of LDI/LA-TOFMS on the vacuum using the first configuration.

was equivalent to the angle of laser incidence on the backside of the thin silver film), and the MCP detector output voltage (which was equivalent to the abundance of an ion). This ASCII file was displayed as a contour plot, as shown in Figure 2.4. It verifies that SPR does assist in the LDI/LA of the thin silver film.

Figure 2.4 Contour plot illustrating a maximum Ag^+ cation intensity at a specific angle of laser incidence during SPR-LDI/LA-TOFMS of a Lumonics thin silver film using the first configuration.

2.1.3 Advantages and Disadvantages

Advantages of this first configuration include the flexibility of using either the 355 or the 532-nm wavelength, and multiple-spot sampling. The chosen wavelength is steered with a right-angle prism external to the ion source chamber of the TOFMS. This prism can be moved closer to or further from the window port, thereby moving the laser beam to provide multiple-spot sampling. Such adaptability of sampling at different spots on the silver film avoids time-consuming analysis (including sample preparation, sample placement in the mass spectrometer, and vacuum roughing to achieve a base pressure of $\sim 1 \times 10^{-6}$ Torr). Multiple-spot sampling can potentially provide multi-sample analysis on a single thin silver film. Since double-field focussing was desirable for TOFMS, grid B, grid C and grid D could be adjusted to change their distances apart. Distances between grids were altered to reduce arcing between the grids, but the mass spectral resolution would be compromised. Consequently the earlier theoretical modelling work in Section 1.3.6 was good to estimate the time of flight, but not sufficient to optimize the resolution.

Modifications to the first configuration of LDI/LA-TOFMS could be easily performed to adopt the SPR mechanism to LDI, as already seen in Figure 2.2. The rotatable mirror could provide a wide range of incident angles for the p-polarized 532-nm wavelength to strike the backside of the thin silver film. However, one unforeseen disadvantage is the inability to inspect where the laser beam strikes on the thin silver film because the tube that grid B was attached to blocked the view. A light bulb could provide illumination inside the vacuum chamber, although proper positioning would be difficult due to two problems. First, the light bulb must be isolated from the high voltages on the

grids and their electrical wires. Second, insufficient lighting power from low bulb wattage did not illuminate the thin silver film surface adequately. Diagnosis of both instrumental and mass spectral problems would be simpler if the location of the laser spot were known.

Another disadvantage of using this first configuration for SPR experiments is the varying laser fluence (mJ/cm^2). With the rotatable mirror, the focusing of the laser beam on the thin silver film was altered during multiple-spot sampling. Also, the position of the focusing lens had to be adjusted when switching between the 355-nm wavelength and the 532-nm wavelength. This was a big inconvenience when comparison of the two wavelengths was constantly made. Unfortunately, a focusing lens was crucial in obtaining a mass spectrum because there was insufficient laser fluence on the thin silver film without one.

A third disadvantage was the difficult and time-consuming process of calibrating the angle of laser incidence. It was essential to calibrate the angle of incidence accurately because the SPR phenomenon could occur at only one particular angle. By recording the stepping numbers when the laser beam traveled at 90° and at 0° angles, all stepping numbers could be converted to angles of incidence. This method of calibration was tedious and not very accurate. Since the 90° and 0° angles were visually monitored, large errors were associated with the angle of incidence ($\pm 2^\circ$). Further errors occurred when multiple-spot sampling was performed. Multiple-spot sampling over 2.5 cm on the thin silver film occurred when the focusing lens in Figure 2.2 was moved into and out of the paper. When the focusing lens moved, the laser path length changed significantly because the laser beam struck the rotatable mirror above or below its previous position.

This varied the angle of incidence on the thin silver film backside by approximately $\pm 2^\circ$. A total error of $\pm 4^\circ$ was too large to make any conclusive statement about the effect of SPR on the LDI/LA process since the theoretical SPR angle using a 532-nm wavelength has a full width at half maximum (FWHM) angle of approximately 1° . Unfortunately, neither the reflectance of the 532-nm laser beam nor the photoacoustic technique could be incorporated into this SPR-LDI/LA-TOFMS configuration. The reflectance route was tried but the 532-nm laser intensity was so great at all angles of incidence that the SPR angle could not be distinguished. The photoacoustic technique was not attempted because if the piezoelectric transducer was glued on to the right-angle prism, the prism would not fit in the aluminum holder. Electromagnetic interference problems would also arise from the electrical wire connections to the transducer. However, these techniques to monitor SPR would still be desirable for the next configurations of the SPR-LDI/LA-TOFMS instrument.

The last disadvantage of this first configuration is the difficulty to perform conventional LDI/LA and MALDI experiments. The current LDI/LA ought to be compared to conventional front-illumination techniques that require the laser beam to strike the front surface of the silver film. The distance between the silver film surface and grid B was very small (~ 2 mm in accordance with the theoretical modeling in section 1.3.6). It was too difficult to manipulate the laser beam to strike the silver film surface because the laser beam would strike the grid B tube unless a hole was made.

Although the above tests were considered preliminary, it was decided that a second configuration would be more desirable to both possibly improve the upper mass

limit of the TOFMS and eliminate many of the above listed disadvantages of this first configuration.

2.2 Second Configuration

2.2.1 Description

The second configuration is a design that was modeled after the SPR-LDI/LA TOFMS system reported previously by Lee et al. (Lee et al., 1992). It was adopted mainly to compare back illumination results with front illumination results. This mass spectrometer was again oriented vertically, as illustrated in Figure 2.5, but had several new features. LDI/LA was accomplished with the only 532-nm wavelength beam of a Nd-Hyper YAG laser. The 532-nm laser beam left the Nd-Hyper YAG laser and traveled through polarization optics to emerge as a p-polarized beam. A thin silver film was coupled to the vertical semi-cylindrical plexiglas prism with diffusion pump oil (Varian Neovac SY). The laser beam passed through this prism, which further focused the laser beam on the backside of the thin silver film to a spot size less than 0.1 mm^2 . A piezoelectric transducer was glued onto the top of the prism to monitor the photoacoustic waves. The electrical wire connections of the piezoelectric transducer were fed to feedthroughs. An external cable connected the electrical feedthroughs with a pre-amplifier. The amplified photo-acoustic signal amplifier was then observed with a DSO. The DSO was either a 100 MHz Gould 465 or the 300 MHz LeCroy 9312M, depending on their availability. The photoacoustic signal was also fed into a boxcar integrator. The amplitude of the photoacoustic signal at a selected delay time was monitored as a function of laser incidence angle in order to plot a SPR angular profile.

A Spellman SL 60PN30 power supply was connected to a high-voltage feedthrough with a high-voltage cable. A thin wire connected the inside of this

Figure 2.5 A Top view of the SPR-LDI/LA-TOFMS instrument in the second configuration. The MCP detector is not shown.

Figure 2.5 B Side view of the SPR-LDI/LA-TOFMS instrument in the second configuration.

feedthrough to a horizontal copper plate, which served as grid A. This copper plate was electrically isolated from the grounded TOFMS with a delrin holder. The semi-cylindrical plexiglas prism was a plexiglas holder attached to a stepping motor (Hurst Model SAS 4004-006) capable of 0.0125 degrees/step. A copper finger was used to hold the thin silver upright film onto the semi-cylindrical prism. The stepping motor provided a variety of angles for the p-polarized 532-nm laser beam to strike the backside of the thin silver film with or without a sample deposited on its surface. One of these angles would be the SPR angle θ_r . A high-voltage power supply (Fluke Model 4088) was connected to the homemade grid controller with a BNC cable. Only the adjustable output voltage B was fed to a MHV feedthrough on the TOFMS with BNC cables to provide an acceleration region. The voltages on the copper plate (or grid A) and grid B provided a non-uniform electrical field around the thin silver film.

2.2.2 New Features

Three new features were added into this second SPR-LDI/LA-TOFMS configuration: a semi-cylindrical plexiglas prism, a high-voltage feedthrough for grid A, and a piezoelectric transducer. The reasons for these additions are given below.

A semi-cylindrical plexiglas prism was easier to calibrate than a right-angle prism. Imagine that a laser beam with a very small diameter that was passed through the semi-cylindrical prism such that the beam emerged at the exact center on the square side of the prism. When the semi-cylindrical prism was rotated, as shown in Figure 2.5A, the laser beam would strike approximately the same spot without any changes in focusing. To perform multiple-spot sampling, the laser beam must be vertically moved. Since the laser beam did not require adjustments, the semi-cylindrical prism provided more precise angle control and calibration.

A high-voltage feedthrough allowed a very large voltage to be applied to grid A. This feedthrough was mandatory because the upper voltage thresholds of BNC, MHV and SHV feedthroughs would not be able to withstand the 10-20 kV needed for increasing the efficiency of the MCP detector, as illustrated in Figure 2.6.

The third addition was a piezoelectric transducer. As mentioned in Section 1.2.5, a piezoelectric transducer could be used to monitor photoacoustic signals, as illustrated in Figure 1.6. In this second configuration, the electrical wires connected to the piezoelectric transducer did not come to the proximity of high voltage wires for the grids. Hence, the photoacoustic output signal was not interfered and such addition of the piezoelectric transducer provided an independent method to monitor SPR.

Figure 2.6 Plot of MCP efficiency against the energy of a positive ion.

2.2.3 SPR-LDI/LA-TOFMS Preliminary Tests

Experiments performed using the second configuration with the 532-nm laser beam passing through a 50-cm focusing lens onto either the front or back side of the thin silver film illustrated no difference between the mass spectra. Only one mass spectral peak signal was observed and this single peak intensity was usually higher than the mass spectral peak intensities observed using the first configuration. The spectrum did not change with any sample applied on the thin silver film. To test the MCP detector, mass spectral peak intensities at constant laser fluence obtained from front illumination experiments were compared for different MCP voltages. As plotted in Figure 2.7, results indicate that the maximum signal intensity appeared at approximately -1.7 kV, as expected from the MCP specifications supplied by Comstock. It was now believed that the difference between the mass spectra from the second configuration and those mass spectra from the first configuration was not due to the method of detection.

Peak times and resolution were not constant because the distances from the LDI/LA spot to the grid B always changed during multiple-spot sampling. Hence, peak assignment to Ag^+ was difficult to make. Front and back illumination experiments performed at identical heights using different grid A (or repeller) voltages illustrated a change in flight time, as shown in Figure 2.8. When a single potential was applied to both the thin silver film and the repeller grid A, the electric field was not uniform although the flight time did not change significantly from before.

It was noticed that the PAS SPR profile magnitude was more pronounced when a higher laser fluence was used. However, the PAS SPR angular profiles for samples

Figure 2.7 Plot of Ag⁺ signal intensity against the voltage applied to the MCP detector using the second configuration.

Figure 2.8 Plot of flight time of Ag⁺ signal against inverse square root of grid A voltage using the second configuration.

deposited on a thin silver film could not be easily distinguished from that for a bare film. Considering the ease of monitoring SPR activity with photoacoustics compared with reflectance methods, PAS measurements were continued.

Both the PAS signal and the mass spectral peak intensity could be monitored simultaneously at the SPR angle by focusing the laser beam onto the backside of the thin silver film. The PAS signal amplitude at the SPR angle decreased as the thin silver film was ablated. This indicated that SPR was lost as soon as ablation of the thin silver film occurred. However, no apparent trend could be established between the mass spectral peak intensity and the PAS signal amplitude. Upon inspection of the thin silver film, holes ablated at and around the SPR angle of $47 (\pm 1)^\circ$ were larger than holes at non-SPR angles.

2.2.4 Advantages and Disadvantages

Advantages of this second configuration include the convenience of front illumination on the silver film, multiple-spot sampling, and accuracy of calibrating the angle of laser incidence. Front illumination experiments, the conventional method of performing LDI/LA-TOFMS, could be compared to back illumination experiments. Multiple spot sampling could be performed, although limited to the vertical direction only for back illumination experiments. The SPR angle was easier to identify by PAS measurements, and angles of laser incidence were easier to calibrate. It was easy to visualize where the laser beam struck the thin silver film which was oriented perpendicular to grid A and grid B. The variation in the laser fluence was eliminated because the focusing was kept constant at any angle of laser incidence.

The biggest disadvantage of this second configuration is the inability to observe a mass spectrum for any sample deposited on the thin silver film. No plausible explanation can be offered except the thought that gas-phase reactions did not occur. This is further described in Section 4.2.2. This disadvantage alone forced another configuration to be developed. Again, arcing between grids was frequently encountered when working with kilo voltages. In addition, this configuration provided a very non-uniform field such that peak reproducibility was not very good and peak identification could not be easily done. Lastly, considering that the plexiglas prism absorbs 355-nm laser light, this configuration would limit the experiments to be performed with the 532-nm laser beam only, unless a quartz semi-cylindrical prism could be afforded. The odd results between our SPR-LDI/LA-TOFMS instrument and the SPR-LDI-TOFMS instrument by Lee et al. (Lee et

al., 1992) may have been expected. It is first speculated that the second excimer laser (308 nm) used by Lee et. al. is crucial for observing a mass spectrum. Unfortunately a second excimer laser and an accurate timing circuit could not be afforded at this time. A second speculation is the choice of metal. Although both silver and aluminum exhibit SPR, silver may not be as chemically reactive towards peptides and proteins as aluminum.

2.3 Third Configuration

2.3.1 Description

Figure 2.9 A shows the LDI/LA-TOFMS in the third configuration. Desorption of either a thin silver film or an organic sample air-dried on the surface of a thin silver film was accomplished with either a p-polarized 532-nm or 355-nm wavelength output beam of a Nd-Hyper YAG laser. The Nd-Hyper YAG laser beam was directed by a right-angle prism located outside the ion source chamber. The laser beam was passed through a focusing lens, traveled through a quartz window port of the ion source chamber and redirected by a translatable prism located inside the ion source chamber. A Spellman SL 60PN30 power supply was connected to a high-voltage feedthrough, which was then connected to an aluminum holder. The aluminum holder was electrically connected to the thin silver film. Hence, the thin silver film served as a repeller plate or grid A. A MHV/SHV feedthrough provided a lower potential to grid B, mounted on an adjustable tube. The high voltage on the thin silver film and the lower voltage on grid B established a uniform electrical field to accelerate the LDI/LA ions towards the MCP detector. After the ions passed through grid B, they flew through an adjustable field-free drift tube that was capped at both ends with two grounded grids C and D. After the ions passed through grid D and left the field-free drift tube, they were slightly post-accelerated towards the MCP detector operated at a front-face voltage of -1.6 kV. The electrical signal from the MCP detector was sent to channel 2 of the 300 MHz LeCroy 9310M DSO (not shown in Figure 2.9). The oscilloscope was triggered at channel 1 by a fast photodiode that was

Figure 2.9 A Top view of the LDI/LA-TOFMS instrument in the third configuration.

measuring a portion of the 532-nm laser beam reflected from the polarization optics.

SPR-LDI-TOFMS was performed easily as illustrated in Figure 2.9 B. The only alterations were the position of the translatable prism and the attachment of a right-angle glass prism to the backside of the thin silver film with diffusion pump oil (Varian Neovac SY). A p-polarized 532-nm laser beam could shine through the right-angle prism to induce SPR activity in the Kretschman geometry.

Figure 2.9 B Top view of the SPR-LDI/LA-TOFMS instrument in the third configuration.

2.3.2 New Features

Three new features were introduced into this third configuration. These are a plexiglas flange covering the top of the ion source chamber (not illustrated in Figure 2.9), a translatable prism and a fast photodiode.

The plexiglas flange provided visual inspection of the thin silver film (or the organic sample deposited on the surface of the thin silver film) during the LDI/LA process. It also provided easy monitoring of electrical and mechanical problems that may occur during an experiment.

The translatable prism was controlled by a stepping motor at a preset/adjustable scanning speed. The translating prism provided multiple-spot sampling. It scanned the laser beam across the thin silver film's backside to generate a sum-averaged LDI/LA-TOFMS spectrum.

A fast photodiode provided a way to monitor the relative laser energy that fell onto the backside of the thin silver film. A correlation could be established between the fast photodiode signal amplitude and the laser energy whenever any physical parameters of the LDI/LA-TOFMS configuration were changed (e.g. removal of the focusing lens). The laser energy was measured with a Scientific Model 365 power/energy meter equipped with a MC250 volume absorbing calorimeter.

2.3.3 (SPR)-LDI/LA-TOFMS Preliminary Tests

The upper mass limit of the third configuration of the LDI/LA-TOFMS instrument appeared to be very similar to that of the first configuration. Experiments with lysozyme, insulin, cytochrome C and bovine serum albumin deposited on a thin silver film all indicated no molecular ion signal, while experiments with gramicidin S (MW 1141 Da) indicated (pseudo) molecular ion signals. Even front-illumination MALDI experiments with these bio-molecules did not give a molecular ion signal. The thin silver film might limit the upper mass limit of this third LDI/LA-TOFMS configuration.

Upon scanning, the resolution of this third LDI/LA-TOFMS configuration did not change compared to the first LDI/LA-TOFMS configuration. However, the signal intensity did increase. This was attributed to fresh areas of the translatable sample being introduced to the laser beam spot. At any laser pulse repetition rate, different scanning speeds would introduce a proportional fresh sample spot. By performing LDI/LA at a slow scanning speed resulted in an introduction of a small fresh spot. By performing LDI/LA at a fast scanning speed resulted in a large fresh spot. This result is illustrated in Figure 2.10 for a gramicidin S sample. Eventually, when the scanning speed was so fast as to expose a fresh spot of sample equal in size to the laser beam for every laser pulse, the sample ion signal intensity would become constant. Clearly, the gramicidin S cation signal has increased by approximately five times at the highest speeds. Since the sample signal intensity increased with high scanning speeds, the upper mass detection limit of the

Figure 2.10 A plot of gramicidin S ion signal against the scanning speed.

Figure 2.11 A plot of melittin ion signal against the scanning speed.

LDI/LA-TOFMS might perhaps increase. This was confirmed with melittin (MW 2846 Da), as illustrated in Figure 2.11. The upper mass limit was increased two fold using the third LDI/LA-TOFMS configuration.

Another approach to increase the upper mass limit of this LDI/LA-TOFMS technique was tested by plating silver on glass microscope slides using the Tollen's reaction. This approach was initially developed because atoms and molecules were known to preferentially desorb from surface defects or sites with low coordination numbers (Dickenson et al., 1994; Hoheisel et al., 1993; Hattori et al., 1991; Vollmer and Trager, 1987; Nishi et al., 1984). It also was developed in hopes of reducing the cost of acquiring Lumonics thin silver films in future experiments. This procedure is described in Section 3.1.1. The preliminary results performed with gramicidin S with scanning demonstrated an increase in the molecular ion signal intensity. The upper mass limit was successfully tested with a poly (ethylene glycol) polymer (MW 6000 Da). These results indicated that the upper mass limit had increased by a factor of approximately two and a half, and together with scanning, by a factor of five.

Under SPR-LDI/LA conditions, lysozyme, insulin, cytochrome C and bovine serum albumin did not exhibit a mass spectrum, even though all samples exhibited a SPR angular profile. However, a larger ablation hole size was observed at the SPR angle of $49 (\pm 1)^\circ$ due to variability in the laser beam focusing. When the prism was translated, the p-polarized 532-nm wavelength laser beam changed drastically from unfocussed conditions to focused conditions, and back to unfocussed conditions. This was confirmed when a maximum Ag^+ cation signal intensity was observed at a specific position along a thin silver film, as illustrated in Figure 2.12. Since the laser fluence (and hence focusing)

Figure 2.12 Trend of Ag^+ cation signal intensity versus position along a thin silver film using the third SPR-LDI/LA-TOFMS configuration.

is the most important parameter in SPR-LDI/LA-TOFMS, further SPR-LDI/LA-TOFMS experiments were not continued.

2.3.4 Advantages and Disadvantages

There are many advantages of the third configuration. Multiple-spot sampling can be performed with ease using either a 355-nm or 532-nm wavelength. Scanning generated mass spectra from numerous laser shots that can be summed and averaged to produce mass spectral peaks with a large S/N without compromising the resolution. Multiple samples can potentially be applied to the silver film surface separately for sequential LDI/LA, consequently increasing the overall time efficiency of LDI/LA-TOFMS analysis. The plexiglas top flange provided visual inspection of the laser spot on the front and backsides of the thin silver film or sample. The laser energy was easily monitored by the fast photodiode. Similar to the first configuration, grid B, grid C and grid D could be adjusted. SPR-LDI/LA-TOFMS was easily adapted to this configuration, although unforeseen complications terminated these experiments. The upper mass limit was improved by a factor of five. The last advantage is the ability to perform both front illumination and back-illumination MALDI experiments of a protein deposited on the surface of a thin silver film. Unfortunately, these results were not encouraging.

There are several disadvantages of this configuration. Firstly, arcing was observed between the grids. Secondly, SPR-LDI/LA-TOFMS experiments could not be performed because constant laser fluence was not obtained. Also, different angles of incidence, required for SPR-LDI/LA-TOFMS analysis was very difficult to attain using the translatable prism. Consequently, modifications to this third configuration would be required, detailed in Chapter 3.

2.4 Fourth Configuration

2.4.1 Description

The fourth configuration is a design that was modeled after the SPR-LDI-TOFMS instrument previously reported by Lee et al (Lee et al., 1992). This mass spectrometer was oriented horizontally, as illustrated in Figure 2.13 A. For LDI/LA-TOFMS experiments, desorption of either a thin silver film or bio-organic molecules air-dried on the surface of a thin silver film was accomplished with either a 532-nm or 355-nm wavelength of a Nd-Hyper YAG laser. The 532-nm wavelength laser beam was directed into the ion source chamber by a right-angle prism located externally from the ion source chamber of the TOFMS. Similarly, the p-polarized 355-nm wavelength laser beam left the Nd-Hyper YAG laser and was directed into the ion source chamber by a right-angle prism located external from the ion source of the TOFMS. The laser beam was passed through a focusing lens and traveled through a quartz window port of the ion source chamber. For back-illumination experiments, the laser beam passed through the glass microscope slide and struck the backside of the thin silver film or a sample deposited on the surface of a thin silver film to a spot size of 0.1 mm^2 . The thin silver film was coupled to a glass microscope slide with diffusion pump oil (Varian Neovac SY), and was electrically isolated from the grounded TOFMS by a delrin holder. For front illumination experiments, the laser beam struck the surface of the thin silver film.

A Spellman SL 60PN30 power supply was connected to a high voltage feedthrough with a high voltage cable. A thin wire connected the inside of the high voltage feedthrough to an aluminum plate, which served as grid A. This aluminum plate

Figure 2.13 A Top view of the LDI/LA-TOFMS instrument in fourth configuration.

was electrically isolated from the grounded TOFMS with a delrin holder. A Fluke high voltage power supply Model 4088 was connected to a homemade grid controller with a BNC cable. This grid controller was a voltage divider. The output voltages (B and C) from the voltage divider were tunable and were fed to the TOFMS with BNC cables. All of these BNC cables were connected to MHV/SHV feedthroughs located at specific areas on the TOFMS. One MHV/SHV feedthrough provided a lower potential from the outside to grid B, mounted on an adjustable tube. A third BNC cable, ~ 5 V, was attached to a MHV/SHV feedthrough (located at the MCP detector), which was connected to grid C. A wire was connected from grid D to the grid C and D tube holder, which, in turn, was connected to ground. The high voltages on the aluminum plate and the lower voltage applied to grid B generated a non-uniform electrical field, while the voltage on grid B and the lower voltage applied to grid C generated a uniform electrical field to accelerate the laser desorbed/ablated ions towards the MCP detector. After the ions have passing through grid B, they drift through an field-free drift tube, capped at both ends with grid C and grid D.

For SPR-LDI/LA-TOFMS experiments, a right-angle glass prism substituted the glass microscope slide, as shown in Figure 2.13 B. Again, a delrin holder supported the right-angle glass prism and isolated the thin silver film from the grounded TOFMS. Hence, only the 532-nm wavelength laser beam traveled through the right-angle glass prism and struck the backside of the thin silver film with or without a deposited sample. By adjusting the angle of incidence with the right-angle prism located external to the ion source chamber, the SPR angle θ_r can be found. The SPR-LDI/LA-TOFMS electronics were identical to the LDI/LA-TOFMS electronics.

Figure 2.13 B End view of the fourth configuration used for SPR-LDI/LA-TOFMS experiments. The aluminum repeller and holder are not shown.

2.4.2 New Features

There was one new feature that has been incorporated into this (SPR)-LDI/LA-TOFMS configuration. This was a plexiglas window port that replaced one of the two quartz window ports of the SPR-LDI/LA-TOFMS instrument of the third configuration. As in the third configuration, the addition of these flanges, combined with the plexiglas flange located at the top of the ion source chamber provided easier visual inspection of the front and back sides of the thin silver film (with or without a deposited sample) during the LDI/LA process or SPR-LDI/LA process. It also provided a method to monitor electrical arcing problems that may occur during an experiment.

2.4.3 (SPR)-LDI/LA-TOFMS Preliminary Tests

A typical back-illumination LDI/LA-TOFMS spectrum of a thin silver film using this configuration illustrated one mass spectral peak. This was identical to the LDI/LA-TOFMS spectrum obtained using the second configuration. Front-illumination LDI/LA-TOFMS experiments of the thin silver film indicated the same results. The LDI/LA-TOFMS spectrum did not change for gramicidin S deposited on the surface of a thin silver film.

Upon performing SPR-LDI/LA-TOFMS experiments of a thin silver film, the single mass spectral signal was a maximum at one angle of incidence. Although the angle of incidence could not be measured with certainty, it was taken to be θ_r . This mass spectral peak was broader, with a $M/\Delta M$ of 2 that did not change with the angle of laser incidence. However, the laser fluence required to generate a mass spectrum at the SPR angle was lower than those for other angles of incidence. Lastly, the same single mass spectral peak was observed when gramicidin S was deposited on the thin silver film, but no molecular ion signal was evident. Compared to the SPR-LDI/LA-TOFMS spectrum of a thin silver film, the resolution was the same and did not change with the angle of laser incidence. However, the lowest laser fluence required to generate a SPR-LDI/LA-TOFMS spectrum occurred at the tentatively assigned θ_r .

Effectively the SPR-LDI/LA-TOFMS instrument of the fourth configuration had an upper mass limit similar to the second configuration. Since the fourth configuration could incorporate our LDI/LA technique and our SPR-LDI/LA technique, it was an

improvement over the second configuration. The flexibility of the fourth configuration provided great insight into the SPR-LDI/LA mechanism, as detailed in Chapter 5.

2.4.4 Advantages and Disadvantages

The preliminary results obtained using this fourth configuration appear to be identical to those obtained in the second configuration. The advantages of this configuration include convenient front illumination of a thin silver film and the multiple-spot sampling. This configuration also has the ability to use either a 532-nm wavelength or a 355-nm wavelength laser beam, although 355-nm wavelength results are not reported in this thesis. The thin silver film is also easy to visually inspect.

The main disadvantage of this configuration is the inability to observe a mass spectrum from any sample deposited on a thin silver film. Additional disadvantages are the difficulty in identifying the angles of incidence for SPR-LDI/LA-TOFMS experiments and the variable laser fluences. Identification of the SPR angle cannot be performed with good accuracy, although the SPR angle can potentially be performed using PAS. This configuration also gives a non-uniform field. Hence, peak identification can not be performed with ease. Lastly, arcing is frequently observed.

2.5 Fifth Configuration

2.5.1 Description

Figure 2.14 A illustrates the orientation of the sample, drift tube, and other essential components of the linear LDI/LA-TOFMS of the fifth configuration. For back-illumination experiments, desorption of either a thin silver film or an organic sample air-dried on the surface of a thin silver film was accomplished with a p-polarized 532-nm laser beam. The s-polarized 532-nm laser beam left the Nd-Hyper YAG laser and traveled through polarization optics to emerge as a p-polarized 532-nm laser beam. A portion of the 532-nm laser beam reflected from the polarization optics was used to trigger the DSO. A right-angle glass prism located external from the ion source chamber of the TOFMS directed the laser beam. The laser beam was directed by another right-angle glass prism mounted on a rotation and translation stage. For LDI/LA-TOFMS, the 532-nm laser beam traveled through a plexiglas window port with diffusion pump oil (Varian Neovac SY). For SPR-LDI/LA-TOFMS experiments, the 532-nm laser beam traveled through a larger right-angle glass prism that was coupled to the outside of a plexiglas flange with diffusion pump oil (Varian Neovac SY), as illustrated in Figure 2.14 B. Either a thin silver film or a sample deposited on the surface of a thin silver film was coupled with diffusion pump oil (Varian Neovac SY) to the inside of a plexiglas flange. The thin silver film with or without a deposited sample was supported with a plastic holder. The laser beam was focused to a spot size of 0.1 mm^2 on the backside of the thin silver film or the backside of a thin silver film with a sample deposited on its surface.

Figure 2.14 A Top view of the LDI/LA-TOFMS instrument in the fifth configuration.

Figure 2.14 B Top view of the SPR-LDI/LA-TOFMS instrument in the fifth configuration.

The front-illumination LDI/LA-TOFMS instrument is illustrated in Figure 2.14 C. A right-angle glass prism located external to the ion source chamber directed either a 532-nm or a 355-nm laser beam. The laser beam was passed through a focusing lens and a quartz window port, to strike the surface of a thin silver film with or without a deposited sample at approximately 45° angle. The laser beam was focused to a spot size of 0.1 mm² on the surface of the thin silver film. The electrical connections were identical to those for the third configuration.

Figure 2.14 C Top view of the front-illumination LDI/LA-TOFMS instrument in the fifth configuration.

2.5.2 New Features

There were two alterations that have been incorporated into this (SPR)-LDI/LA-TOFMS instrument. The first was a plexiglas window port that held the thin silver film. As in the third configuration, the thin silver film had the high voltage. However, the drift tube had to be placed nearer to the thin silver film (or grid A). An additional aluminum tube was added so that there was approximately 3 cm difference between the grid A and grid B. Hence, the distance between grid D and the MCP detector was now longer. The second alteration was the coupling of the thin silver film to the plexiglas window port. This was performed to incorporate the SPR phenomenon easily into the LDI/LA-TOFMS instrument.

As previously described in this chapter, the SPR-LDI/LA-TOFMS instrument of previous configurations was not very successful due to difficulties in measuring the SPR angle. With this configuration, the angles could be calibrated and measured easily because a new rotational and translational stage was added. This is the only new feature that was incorporated into the fifth, and final configuration.

2.5.3 (SPR)-LDI/LA-TOFMS Preliminary Tests

Preliminary experiments with the LDI/LA-TOFMS instrument of the fifth configuration demonstrated bigger limitations than the LDI/LA-TOFMS instrument of the third configuration. Large molecular weight proteins, like horse heart cytochrome C, hen egg white lysozyme and bovine serum albumin could not be detected. In fact, even melittin could not be detected with the fifth configuration. Front-illumination experiments showed the same results. MALDI experiments were performed to try to increase the upper mass limit of this (SPR)-LDI/LA-TOFMS instrument, but these were also unsuccessful.

However, most of the drawbacks of the previous SPR-LDI/LA-TOFMS instruments were overcome. The angles of incidence could be measured with an accuracy of approximately $\pm 1^\circ$, significantly better than the previous configurations. Results obtained from multiple-spot sampling tests were reproducible. The difference in resolution measured between a LDI/LA-TOFMS and SPR-LDI/LA-TOFMS spectrum was identical. While mass spectra were recorded, the laser fluence at one angle of incidence was easily monitored, even though it changed with a different angle of incidence. The results of these experiments are further detailed in Chapter 5.

2.5.4 Advantages and Disadvantages

The preliminary results obtained in this configuration are similar to those obtained in the third configuration. The advantages of this configuration include the front illumination of a thin silver film and the multiple spot sampling. The method used to marry the SPR phenomenon with the LDI/LA-TOFMS was accomplished successfully. Minor calibration work was required for determining an angle of incidence. The thin silver film was also easy to visually inspect.

The main disadvantage of this configuration was the inability to observe a mass spectrum for a sample with a large molecular weight. In fact, the upper mass limit for the fifth configuration was lower than that for the third configuration. Consequently, LDI/LA-TOFMS experiments were performed using the third configuration, while SPR-LDI/LA-TOFMS experiments were performed with the fifth configuration. The upper mass limit of the SPR-LDI/LA-TOFMS instrument appeared to be limited by the inability to scan, and the thickness of a thin silver film. Back-illumination experiments could only be performed with the 532-nm laser beam, while front-illumination experiments could accommodate the 532-nm and 355-nm laser beam. Consequently, results presented in Chapter 3 and 4 were primarily obtained from the third configuration, and results presented in Chapter 5 were obtained from the fifth configuration. Lastly, as observed in the previous configurations, arcing was frequently observed.

In summary, since arcing occurred in all of the configurations, the results presented henceforth were obtained from modifications of the third, fourth and fifth configurations. Although the theoretical modeling of the double-focusing system could

predict the time-of-flight of an ion for our (SPR)-LDI/LA-TOFMS, the distances s and d could not be used. Consequently, different operational settings of each configuration were used to reduce the risk of arcing and produce results that were reproducible

Chapter 3: LDI/LA-TOFMS Results and Discussion

3.1 Thin Silver Films

3.1.1 Experimental Details

To perform any mass spectral analysis, a background must be obtained and correctly identified. Using this LDI/LA-TOFMS technique, the background spectrum was measured from a thin silver film without a sample. Back-illumination LDI/LA-TOFMS spectra were summed and averaged over at least 50 laser shots to improve the signal-to-noise ratio, while front-illumination LDI/LA-TOFMS spectra were each measured in a single-shot. A signal-to-noise ratio greater than 10:1 was adopted as the criterion for a mass spectral peak.

Back-illumination LDI/LA-TOFMS spectra of Lumonics and Tollen's thin silver films were obtained using the third configuration (refer to Figure 2.9 A). Front-illumination LDI/LA-TOFMS spectra of a Lumonics thin silver film was also obtained using the fifth configuration (refer to Figure 2.14 C). The parameters set on the LDI/LA-TOFMS instrument are listed in Table 3.1.

The Lumonics Optics Group (Nepean, ON) prepared thin silver films by thermal evaporation of 99.9% pure silver at a base pressure of 10^{-7} torr for deposition on one clean face of microscope glass slides. Their nominal thickness was given by a quartz crystal oscillator in the evaporation system to be 40 nm. These silver-coated glass slides were stored in a vacuum dessicator until use.

Table 3.1. Operational settings of the LDI/LA-TOFMS instrument.

Parameter	Value (Third Configuration)	Value (Fifth Configuration)
Pressure P (torr)	1.5×10^{-6}	$0.25\text{--}1.5 \times 10^{-6}$
Laser wavelengths (nm)	355 and 532	355 and 532
Laser fluence (mJ/cm^2)	minimum	minimum
Laser repetition rate (Hz)	10	1
Laser pulse width at FWHM (ns)	8	8
Lens focal length (cm)	20	50
Drift tube length D (cm)	42.2	42.2
Length s (cm)	1.0	0.4
Length d (cm)	-	2.1
Grid a voltage (kV)	20	10
Grid b voltage (kV)	0	9.6
Grid c voltage (kV)	0	0
Front-face voltage on MCP (kV)	-1.6	-1.6

Thin silver films were also prepared in our laboratory using the classical Tollen's chemistry. Microscope glass slides and a Pyrex petri dish were first cleaned with acetone, soaked in 100% HNO_3 for two days, boiled in the HNO_3 for one hour, and rinsed with distilled deionized water. In the clean petri dish, 1 mL of 10% NaOH was added to 40 mL of 5% AgNO_3 to produce the brown Ag_2O precipitate. The solution was diluted with 150 mL distilled deionized water before the precipitate was dissolved in a minimum amount (~ 3 mL) of 50% NH_4OH , added dropwise while the solution was being stirred. Then 0.1 mL of benzaldehyde was added, and the reaction mixture was quickly stirred. Several clean glass slides were positioned in this Tollen's reagent solution for silver plating after the petri dish was placed on a hot plate set at 50°C . At various times, the silver-coated glass slides were removed from the plating solution and rinsed with distilled deionized water. After drying in air, these silver-plated glass slides were stored in a vacuum dessicator until use. Before these glass slides were used, each

was analyzed by UV-visible spectrophotometry to determine its silver film thickness.

The Tollen's reagent solution on standing has the capability of forming silver fulminate ($\text{AgC}_2\text{N}_2\text{O}_2$), which is a violently explosive compound. Therefore, the Tollen's reagent solution was prepared immediately before use and disposed of into a waste container with plenty of water immediately after plating.

3.1.2 Mass Spectral Analysis

The third configuration LDI/LA-TOFMS spectra obtained for a Lumonics thin silver film using a 532-nm and a 355-nm laser beam is shown in Figure 3.1. In both spectra, three consistent peaks are observed. These peaks are assigned as the Ag^+ , Ag_2^+ , and Ag_3^+ cation signals. Similar mass spectral peaks corresponding to gold and gold clusters ($[\text{Au}_n]^+$ for $1 \leq n \leq 3$) had previously been reported when performing LDI-TOFMS with a 355-nm wavelength (Mouradian, et al., 1996). The TOFMS spectrum obtained using a 532-nm laser beam illustrates a relative intensity of approximately 100:10:20 for the $\text{Ag}^+ : \text{Ag}_2^+ : \text{Ag}_3^+$ cations. However, the TOFMS spectrum obtained using a 355-nm wavelength laser beam illustrates a relative intensity of approximately 100:50:10 for the $\text{Ag}^+ : \text{Ag}_2^+ : \text{Ag}_3^+$ cations. The laser pulse energy did not affect the relative intensity of the cluster cations. Larger silver cluster cations ($n \geq 4$) are present in the mass spectra as weak signals. In addition, two peaks are sometimes observed, which correspond to a Na^+ cation signal and a Ca^+ cation signal. These cations are thought to originate from the glass microscope slide.

The third configuration LDI/LA-TOFMS spectrum obtained for a Tollen's thin silver film using a 532-nm laser beam is shown in Figure 3.2. A large Na^+ cation signal, thought to originate from the microscope glass slide, is seen in the spectrum. Also seen at somewhat lower intensities are $[\text{Ag}_n]^+$ ($n=1,3$) cation signals and a Ca^+ cation signal. A relative intensity of 100:60:75 is illustrated for the $\text{Ag}^+ : \text{Ag}_2^+ : \text{Ag}_3^+$ cations. Apparently the relative intensity from a Tollen's thin silver film is different than that from a Lumonics thin silver film. This is most likely due to differences in the thin silver film

Figure 3.1 Third configuration LDI/LA-TOFMS spectra of a Lumonics thin silver film using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

Figure 3.2 The 532-nm LDI/LA-TOFMS spectrum of a Tollen's thin silver film.

thickness and surface morphology. Since preferential desorption of atoms and molecules occur from defects on surfaces (sites with low coordination numbers) (Dickenson et al., 1994; Hoheisel et al., 1993; Hattori et al., 1991; Vollmer and Trager, 1987; Nishi et al., 1984), it is not unreasonable to observe $[\text{Ag}_n]^+$ cations at different relative intensities between Lumonics and Tollen's thin silver films. X-ray qualitative and quantitative analysis on a scanning electron microscope (SEM) of all Tollen's thin silver films indicated an abundance of silver atoms. Hence, reagent chemicals other than silver used

in the preparation of Tollen's thin silver films did not contribute significantly towards mass spectral analysis.

The fifth configuration LDI/LA-TOFMS spectra obtained for a Lumonics thin silver film using a 532-nm and a 355-nm wavelength laser beam are shown in Figure 3.3. The Na^+ peak is seen at m/z 23. Three major silver cation peaks, $[\text{Ag}_n]^+$ ($1 \leq n \leq 3$), are seen at m/z 108, 216 and 323 in the 532-nm spectrum. A relative intensity of 100:25:60 for the $\text{Ag}^+ : \text{Ag}_2^+ : \text{Ag}_3^+$ cations is illustrated. A similar 355-nm spectrum shows the silver cations at m/z 108, 216 and 323 as the dominant features. Other cations are seen at m/z 23 and 40, corresponding to Na^+ and Ca^+ cations.

Although the Na^+ and Ca^+ cations are observed in most of the mass spectra, they seem to appear randomly. However, the silver cluster cations, $[\text{Ag}_n]^+$, are thought to be the product of chemical ionization of neutral silver particles $[\text{Ag}_{n-1}]$ by a Ag^+ cation via gas-phase ion-molecule reactions, further described in Section 4.2.2. The direction of laser incidence (back illumination vs. front illumination) does not affect the formation of the silver cluster cations. These peaks are expected to be present in all subsequent mass spectra and in large abundance. Further experimentation must be performed to verify if the cations are formed in an electronic excitation mechanism (where an electron is excited to an anti-bonding orbital (Gotz et al., 1996 A) or a thermal mechanism. If an electronic excitation mechanism is apparent, our LDI/LA technique is soft and will not fragment sample molecules to a large extent. However, a thermal mechanism will fragment sample molecules to a larger extent that can make mass spectral interpretation very difficult. Results will be elaborated in Section 4.2.3.

Figure 3.3 Fifth configuration LDI/LA-TOFMS spectra of a Lumonics thin silver film using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

3.2 Bio-organic Compounds

3.2.1 Experimental Details

To evaluate the chemical reactivity of silver towards compounds via gas-phase ion-molecule reactions (see Section 4.2.2), a variety of bio-organic compounds were tested with the back-illumination LDI/LA technique. Approximately 50 mass spectra were summed and averaged to improve the signal-to-noise ratio. A mass spectral peak was recognized when its signal-to-noise ratio was greater than 5:1. Since the mass of each bio-organic compound was known, a silver cation attached to the bio-organic compound was readily identified.

Back-illumination LDI/LA-TOFMS spectra of bio-organic compounds deposited on a thin silver film were obtained using the third configuration (refer to Figure 2.9 A). The parameters set on the LDI/LA-TOFMS instrument are listed in Table 3.2.

Bio-organic compounds that were analyzed are listed in Table 3.3. The molecular structures of the organic compounds are shown in Figure 3.4. Each sample was prepared by deposition of 50 μ l of a given concentration in a suitable solvent on the surface of a 2.5 cm x 2.5 cm thin silver film. Air drying evaporated the solvent from the sample to form a deposited sample with non-uniform thickness. Each sample was analyzed via back illumination with either the 532-nm wavelength or the 355-nm wavelength to produce a LDI/LA-TOFMS spectrum.

Table 3.2. Operational settings of the LDI/LA-TOFMS instrument.

Parameter	Value
Pressure P (torr)	1.5×10^{-6}
Laser wavelengths (nm)	355 and 532
Laser fluence (mJ/cm^2)	minimum
Laser repetition rate (Hz)	10
Laser pulse width at FWHM (ns)	8
Lens focal length (cm)	20
Drift tube length D (cm)	42.2
Length s (cm)	1.0
Length d (cm)	-
Grid a voltage (kV)	20
Grid b voltage (kV)	0
Grid c voltage (kV)	0
Front-face voltage on MCP (kV)	-1.6

Table 3.3. Bio-organic compounds, their concentration and solvent used for LDI/LA-TOFMS analysis at the chosen wavelength.

Bio-organic Compounds	Concentration	Solvent	Wavelength
Dithizone (MW 244)	15.0 mg/ml	Carbon tetrachloride	355 nm
1,4,8,11 – Tetraazocyclotetradecane (MW 200)	4.0 mg/ml	Ethanol	355 nm
Dicyclohexyl-18- crown-6 ether (MW 373)	4.0 mg/ml	Methanol	355 nm
Dendrimer (MW 1342)	4.0 mg/ml	Chloroform	355 nm
Gramicidin S (MW 1141)	4.0 mg/ml	Methanol	532 nm
Substance P (MW 1347)	4.0 mg/ml	Methanol	355 nm
Melittin (MW 2846)	4.5 mg/ml	Methanol	532 nm

Figure 3.4 Structures of organic compounds analyzed using the third configuration of the LDI/LA-TOFMS instrument.

3.2.2 Organic Molecules

Dithizone is an efficient organic ligand that binds to a silver cation in aqueous solution below pH 7 to form a neutral complex in classical solvent extraction. Its attraction to the silver cation is attributed to a lone pair of electrons on the sulfur atom (Nabeshima et al., 1996). Hence, this compound was chosen to test the chemical reactivity of a Ag^+ cation with the sulfur atom in the gas phase. A dithizone sample deposited on a thin silver film produced a LDI/LA-TOFMS spectrum exhibited no molecular ion M^+ signal. However, a moderate $[\text{M}+\text{Ag}]^+$ adduct cation signal was observed, as illustrated in Figure 3.5. This result confirms the chemical reactivity of a Ag^+ cation in the gas phase with a sulfur atom.

1,4,8,11-Tetraazocyclotetradecane is an ionophore that forms a tight complex with silver in aqueous solution (Grzejdziak, 1994). This compound was chosen to test the chemical reactivity of a Ag^+ cation towards lone electron pairs of nitrogen atoms in the gas phase. Its LDI/LA-TOFMS spectrum is shown in Figure 3.6. A good signal intensity is observed for a $[2\text{M}+\text{Ag}]^+$ cation. However, the $[\text{M}+\text{Ag}]^+$ and M^+ cation signals are not observed. These results suggest that a Ag^+ cation is sandwiched between two neutral 1,4,8,11-tetraazocyclotetradecane molecules to form a stable structure in the gas phase. The Ag^+ cation is probably electrostatically bound to the eight lone pairs of electrons on the four nitrogen atoms.

Dicyclohexyl-18-crown-6 is a cyclic crown ether containing six regularly spaced oxygen atoms. The lone electron pairs of the oxygen atoms form a cavity that can bind a Ag^+ cation to form a stable coordination complex. This compound was chosen to test

Figure 3.5 LDI/LA-TOFMS spectrum of dithizone (m/z 244) using a 355-nm laser beam.

Figure 3.6 LDI/LA-TOFMS spectrum of 1,4,8,11-tetraazocyclotetradecane (m/z 200) using a 355-nm laser beam.

whether oxygen would exhibit the same Ag^+ chemical reactivity as sulfur and nitrogen. Its LDI/LA-TOFMS spectrum is shown in Figure 3.7. A strong M^+ cation signal is observed, accompanied with a weak $[\text{M}+\text{Ag}]^+$ cation signal. The lone electron pairs on the oxygen atoms and the cyclic structure play an important role in the gas-phase ion chemistry of the Ag^+ cation (Nabeshima et al., 1996).

A new dendrimer ($\text{C}_{87}\text{H}_{63}\text{O}_{12}\text{N}_3$), which was synthesized from [5]-helicene dicarboxylic anhydride, was acquired to test the versatility of this LDI/LA-TOFMS technique. Since silver cationized five phenylacetylene dendrimers had been reported (Walker et al., 1994), this dendrimer might behave similarly and extend the upper mass limit of organic molecules that could be analyzed with the present technique. The lone electron pairs on the oxygen and nitrogen atoms, and the extensive π electron conjugation in the carbon rings might provide a binding site for the Ag^+ cation. The LDI/LA-TOFMS spectrum is shown in Figure 3.8. A weak M^+ cation signal is observed and it is accompanied by a weaker $[\text{M}+\text{Ag}]^+$ cation signal. These results demonstrate the ability of this LDI/LA technique to determine the molecular weight of dendrimers without the addition of a MALDI matrix.

Figure 3.7 LDI/LA-TOFMS spectrum of dicyclohexyl-18-crown-6 ether (m/z 373) using a 355-nm laser beam.

Figure 3.8 LDI/LA-TOFMS spectrum of a dendrimer ($C_{87}H_{63}O_{12}N_3$) (m/z 1342) using a 355-nm laser beam.

3.2.3 Bio-organic Molecules

Gramicidin S is a cyclic decapeptide that plays an important ionophorous role in biochemical ion balance processes. Although the formation of protonated gramicidin S had been previously reported for substrate-assisted LD/CI off thin metal films (Speir and Amster, 1992), the formation of Li^+ , Na^+ , and K^+ cationized gramicidin S had also been observed (Wang et al., 1993; McIver et al., 1994; Gross and Williams, 1996). The variety of functional groups and its cyclic structure could provide binding sites for the Ag^+ cation. This compound was chosen to test the chemical reactivity of Ag^+ towards a bio-molecule. Its LDI/LA-TOFMS spectrum is shown in Figure 3.9. A weak $[\text{M}+\text{Na}]^+$ cation signal is observed and it is accompanied by a weaker $[\text{M}+\text{Ag}]^+$ cation signal. The chemical reactivity of Ag^+ with the functional groups in a cyclic structure of gramicidin S is thus demonstrated.

Substance P is a linear peptide that required the addition of MALDI matrix α -cyano-4-hydroxycinnamic acid to generate a molecular cation signal (Vorm et al., 1994). However, the variety of functional groups in this bio-molecule could provide binding sites for the Ag^+ cation. Its LDI/LA-TOFMS spectrum is shown in Figure 3.10. A weak M^+ cation signal is observed and it is accompanied by a stronger $[\text{M}+\text{Ag}]^+$ cation signal. These results lead to three conclusions. Firstly, the chemical reactivity of Ag^+ towards a functional group along the backbone of this small peptide is evident. Secondly, a cyclic structure is not mandatory for successful cationization of a bio-molecule with a Ag^+ cation. Lastly, a MALDI matrix is not required for analyzing substance P using the

Figure 3.9 LDI/LA-TOFMS spectrum of gramicidin S (m/z 1141) using a 532-nm laser beam.

Figure 3.10 LDI/LA-TOFMS spectrum of substance P (m/z 1347) using a 355-nm laser beam.

present LDI/LA technique, even though the linear peptide has a facile backbone (Becker and Wu, 1995).

Melittin is a larger linear peptide than substance P, and it is found naturally as bee venom. Since intact peptide molecular ions above 2000 Da are difficult to produce using the conventional LDI technique (Vorm et al., 1994), this bio-molecule was chosen to further establish the upper mass limit of LDI/LA-TOFMS. Its LDI/LA-TOFMS spectrum is shown in Figure 3.11. A weak M^+ cation signal is observed and it is accompanied by a stronger $[M+Ag]^+$ cation signal. These results demonstrate the successful Ag^+ cationization of a peptide with molecular weight greater than 2800 Da. The 532-nm wavelength probably transfers lower photon energy to the sample during LDI/LA than a 355-nm wavelength. Consequently, the internal energy of the sample is lowered, there is little fragmentation, and a M^+ cation signal is hence observed. However, the detection of larger peptides is limited because the binding energy of these peptides to the silver film surface will increase. This is expected because larger peptides have more polar functional groups. Also, the interaction between individual peptides will increase, which can result in a larger laser energy input required for LDI/LA. Consequently, the internal energy per bond can become so high as to cause the peptide's fragmentation.

Figure 3.11 LDI/LA-TOFMS spectrum of melittin (m/z 2846) using a 532-nm laser beam.

3.3 Polyaromatic Hydrocarbons (PAHs) and Fullerene Compounds

3.3.1 Experimental Details

To further illustrate that gas-phase ion/molecule reactions occurred using our LDI/LA technique (see Section 4.2.2), mass spectral analysis was performed on PAH and fullerene compounds whose chemical reactivity toward silver had been recently reported (Ng et al.: 1998). Over 200 mass spectra were summed and averaged to obtain a signal-to-noise ratio greater than 5:1 for the molecular ion peak/signal.

Back-illumination LDI/LA-TOFMS spectra of polycyclic aromatic hydrocarbons (PAH) and fullerene compounds (50 μ l) deposited on a thin silver film were obtained using the third configuration (refer to Figure 2.9A). Two modifications were made to reduce the effect of fluctuations in the laser fluence. The sample was put on a lateral translating stage. Also, the focusing lens and the right angle prism were placed outside the ion source chamber so that laser beam was focused on the silver film. The parameters set on the LDI/LA-TOFMS instrument are listed in Table 3.4. The PAH compounds, listed in Table 3.5, and the fullerene compounds, listed in Table 3.6, were analyzed. Their structures are illustrated in Figure 3.12.

Table 3.4. Operational settings of the LDI/LA-TOFMS instrument.

Parameter	Value
Pressure P (torr)	1.5×10^{-6}
Laser wavelengths (nm)	355 and 532
Laser fluence (mJ/cm^2)	minimum
Laser repetition rate (Hz)	10
Laser pulse width at FWHM (ns)	8
Lens focal length (cm)	20
Drift tube length D (cm)	42.2
Length s (cm)	1.0
Length d (cm)	-
Grid a voltage (kV)	20
Grid b voltage (kV)	0
Grid c voltage (kV)	0
Front-face voltage on MCP (kV)	-1.6

Table 3.5. PAH compounds, their concentrations, and the sample preparation solvent used for LDI/LA-TOFMS analysis.

Compound	Concentration	Solvent
Anthracene (MW 178)	4.0 mg/ml	Benzene
Phenanthrene (MW 178)	4.0 mg/ml	Benzene
Anthrone (MW 194)	8.0 mg/ml	Toluene
9,10-Anthraquinone (MW 208)	4.0 mg/ml	Toluene
9,10-Phenanthroquinone (MW 208)	4.0 mg/ml	Diethyl ether
Pyrene (MW 202)	4.0 mg/ml	Toluene
Triphenylene (MW 228)	4.0 mg/ml	Toluene
Chrysene (MW 228)	4.0 mg/ml	Toluene
Benz[a]anthracene (MW 228)	4.5 mg/ml	Toluene
o-Terphenyl (MW 230)	4.0 mg/ml	Benzene
p-Terphenyl (MW 230)	4.0 mg/ml	Benzene
Benzo[e]pyrene (MW 252)	4.0 mg/ml	Toluene
Benzo[a]pyrene (MW 252)	4.0 mg/ml	Toluene
Perylene (MW 252)	4.0 mg/ml	Toluene
Coronene (MW 300)	4.0 mg/ml	Toluene

Table 3.6. Fullerene compounds, their concentrations, and the sample preparation solvent used for LDI/LA-TOFMS analysis.

Compounds	Concentration	Solvent
C60 (MW 720)	4.0 mg/ml	Toluene
C70 (MW 840)	4.0 mg/ml	Toluene
1,2-(Dimethoxymethano) fullerene C60 (MW 795)	4.0 mg/ml	Toluene
Tert-butyl (1,2- methanofullerene C60) – 61 – carboxylate (MW 835)	4.0 mg/ml	Toluene

Ring Letters

E,A,B

D,A,B

A,B,C,D

A,B,D,F

D,C,B,H

D,A,B,H

A,B,C,D,F

A,B,C,D,H

F,G,A,D,C

A,B,C,D,E,F,G

Compound

Anthracene

Phenanthrene

Pyrene

Triphenylene

Chrysene

Benz[a]anthracene

Benzo[e]pyrene

Benzo[a]pyrene

Perylene

Coronene

Letter

A

B

Compound

o-Terphenyl

p-Terphenyl

Anthrone

9,10-Anthraquinone

9,10-Phenanthroquinone

Figure 3.12 Structures of PAH compounds analyzed using the third configuration of the LDI/LA-TOFMS instrument.

3.3.2 Three-Ringed PAH and Related Compounds

Cation-aromatic π interactions have been suggested as playing important roles in the molecular recognition of many biological and macromolecular systems (Ma and Dougherty, 1997; Kim et al., 1994 A). The quantitative measurement of cation affinity of neutral molecules in the gas phase can reveal the fundamental nature of cation-aromatic π interactions (Ng et al., 1998). Cation-aromatic π interactions have been investigated for $[(C_6H_6)_n+Au]^+$ and $[(C_6H_6)_n+Al]^+$ ($n=1,2$) using a LA-molecular beam method (Higashide et al., 1990), while silver cationized poly(styrene) and poly(vinyl-naphthalene) also showed π -bonded complex structures in TOF-SIMS studies (Pleul et al., 1997). Apparently silver cations can bind with delocalized π electrons, like cyclopentadienyl-(triphenylphosphine)silver(I) that has a π -bonded pentahapto cyclopentadienyl structure based on IR and NMR spectra (Hofstee et al., 1976), and localized π electrons, like the $[Ag-C_2H_4]^+$ cation (Nishigaki et al., 1979). In this work, an attempt is made to elucidate the nature of cation-aromatic π interactions with PAH compounds using our LDI/LA technique.

The LDI/LA-TOFMS spectra of anthracene and phenanthrene revealed no M^+ or $[M+Ag]^+$ peak. Only Na^+ , Ca^+ and $[Ag_n]^+$ ($1 \leq n \leq 3$) peaks were observed in the mass spectra. The absence of a M^+ cation was expected because a 249-nm wavelength would be required for multi-photon ionization of anthracene (Egorov et al., 1984). Since the molecular weight of anthracene and phenanthrene is only 178 Da, sublimation loss of both samples could also occur inside the ion source chamber under vacuum.

By substituting oxygen on the central ring of anthracene to form anthrone, the molecular weight increases to 194 and sublimation loss becomes less likely. Also, a dipole is present, which could promote electrostatic interactions between anthrone and the Ag^+ cation. However, the LDI/LA-TOFMS spectrum of anthrone only revealed the Na^+ , Ca^+ and $[\text{Ag}_n]^+$ ($1 \leq n \leq 3$) peaks. Apparently the $\text{C}=\text{O}$ dipole was not strong enough to attract a silver cation for chemical ionization, even though a great electron density lies on the oxygen.

Similar LDI/LA-TOFMS spectra were observed for 9,10-anthraquinone and 9,10-phenanthroquinone. The additional oxygen atom did not enhance the molecule's binding with Ag^+ cation via electrostatic interactions, regardless of the presence or absence of a net dipole from the $\text{C}=\text{O}$ groups. Since a M^+ peak for 1,4,8,11-tetraazocyclotetradecane (MW 200) was detected in the previous section, sublimation loss of 9,10-anthraquinone and 9,10-phenanthroquinone (MW 208) should not be a key factor. Clearly, the absence of the M^+ and $[\text{M}+\text{Ag}]^+$ in these LDI/LA-TOFMS spectra must be the result of the ionization process and the specific chemical reactivity of the silver cation, as detailed in Section 3.3.6. However, Ag^+ complexation with larger PAH compounds involving delocalized π bonding was further investigated.

3.3.3 Four-Ringed PAH Compounds

The 532-nm LDI/LA-TOFMS spectrum of pyrene did not reveal any M^+ or $[M+Ag]^+$ peak. However, the 355-nm LDI/LA-TOFMS spectrum of pyrene exhibited a small $[M]^+$ signal at m/z 202, as illustrated in Figure 3.13. Clearly the introduction of a fourth ring to the phenanthrene structure enhanced the ionization of pyrene.

The 532-nm LDI/LA-TOFMS spectrum of triphenylene did not reveal any M^+ or $[M+Ag]^+$ peak. However, strong M^+ and $[M+Ag]^+$ signals were exhibited in the 355-nm LDI/LA-TOFMS spectrum of triphenylene, as illustrated in Figure 3.14. Apparently the position of the fourth ring determines whether the $[M+Ag]^+$ cation is formed readily within the ablation plume. The binding site is generally determined by the location of the greatest electron density. Triphenylene has the greatest electron density on its central ring in our preliminary computer modeling. Apparently the electron contributions from the outermost rings are large enough to substantially raise the electron density on the central ring, thereby providing an effective site for Ag^+ binding via cation- π interaction and resulting in the formation of $[M+Ag]^+$.

The 532-nm LDI/LA-TOFMS spectrum of chrysene did not reveal any M^+ or $[M+Ag]^+$ signals. However, strong M^+ and $[M+Ag]^+$ signals were exhibited in the 355-nm LDI/LA-TOFMS spectrum of chrysene, as illustrated in Figure 3.15. Since the greatest electron density lies on the two central rings, the Ag^+ cation likely resides on them.

The 532-nm and 355-nm LDI/LA-TOFMS spectra of 1,2-benz[a]anthracene illustrated the presence of the M^+ cation, as shown in Figure 3.16. An additional

Figure 3.13 LDI/LA-TOFMS spectrum of pyrene (m/z 202) using a 355-nm laser beam.

Figure 3.14 LDI/LA-TOFMS spectrum of triphenylene (m/z 228) using a 355-nm laser beam.

Figure 3.15 LDI/LA-TOFMS spectrum of chrysene (m/z 228) using a 355-nm laser beam.

$[M+Ag]^+$ signal is observed in the 355-nm LDI/LA-TOFMS spectrum. Apparently the M^+ cation is formed more readily than the previous PAH compounds using either the 532-nm or 355-nm wavelength. Similar to triphenylene, the Ag^+ cation likely resides on ring A because this is the location with the greatest electron density, as determined in our preliminary computer modeling.

Figure 3.16 LDI/LA-TOFMS spectra of benz[a]anthracene (m/z 228) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

3.3.4 Silver Cation Affinity toward Terphenyl Compounds

The Ag^+ cation affinity toward terphenyl compounds is expected to be different compared to the previous PAH compounds analyzed. The terphenyl structures have the greatest electron density on their outer benzene rings where silver cationization should occur. Also, these rings can rotate and their relative position can change the dipolar electrostatic interactions (o-terphenyl has a larger dipole than p-terphenyl) with Ag^+ . It should be emphasized that these compounds do not absorb the 532-nm and 355-nm wavelengths strongly. Silver cationized products are therefore expected to occur via gas-phase reactions.

The 532-nm LDI/LA-TOFMS spectrum of o-terphenyl illustrated the absence of the M^+ and $[\text{M}+\text{Ag}]^+$ cations. However, as shown in Figure 3.17, the 355-nm LDI/LA-TOFMS spectrum of o-terphenyl indicated two strong signals at m/z 338 and m/z 128. The first strong peak corresponds to the $[\text{M}+\text{Ag}]^+$ cation, while the second one corresponds to a fragment cation. Another smaller signal at m/z 230 corresponds to the M^+ cation. Since most of the electron density lies on the two outer rings to form a small dipole within the o-terphenyl structure, the Ag^+ cation resides either on one of the outer benzene rings to form the $[\text{M}+\text{Ag}]^+$ cation, or between the two outer benzene rings in a sandwich structure.

The 532-nm LDI/LA-TOFMS spectrum of p-terphenyl illustrated the absence of the M^+ and $[\text{M}+\text{Ag}]^+$ cations. Similar to the o-terphenyl, the 355-nm LDI/LA-TOFMS spectrum of p-terphenyl had two peaks at m/z 338 and m/z 128, as shown in Figure 3.18. These peaks correspond to the $[\text{M}+\text{Ag}]^+$ cation and the fragment cation, respectively.

Figure 3.17 LDI/LA-TOFMS spectrum of o-terphenyl (m/z 230) using a 355-nm laser beam

Figure 3.18 LDI/LA-TOFMS spectrum of p-terphenyl (m/z 230) using a 355-nm laser beam.

The absence or very low abundance of the M^+ peak indicates the stability of the $[M+Ag]^+$ cation. The Ag^+ cation is probably located on one of the outer benzene rings that have the greatest electron density.

To investigate the silver cation affinity toward these two terphenyls, the relative $[M+Ag]^+$ cation signals must be compared (further detailed in Section 4.1.3). These signals must be normalized relative to the Ag^+ cation signal. The $[M+Ag]^+/Ag^+$ ratios for o-terphenyl and p-terphenyl were calculated to be 0.6 ± 0.2 and 0.2 ± 0.2 , respectively. This result means that if the same Ag^+ concentration is available to both terphenyls, the o-terphenyl compound will have a higher Ag^+ cation affinity. It is speculated that the higher Ag^+ affinity toward the o-terphenyl molecule is primarily due to its stronger dipole and greater stability of the resulting sandwich structure.

Conventional EI mass spectrometry illustrated more fragmentation of o-terphenyl than p-terphenyl, which was attributed to the steric hindrance between the two outer benzene rings that destabilizes the o-terphenyl molecule (Gallegos, 1967). To investigate the extent of fragmentation for both $[M+Ag]^+$ cations using our technique, the $[M+Ag]^+/[fragment]^+$ ratio must be examined. The $[M+Ag]^+/[fragment]^+$ ratios for o-terphenyl and p-terphenyl were calculated to be 1.1 ± 0.2 and 1.2 ± 0.2 , respectively. These ratios are close to each other, indicating similar fragmentation extents; they are approximately one, suggesting similar stability between the $[M+Ag]^+$ cations and their fragments. The Ag^+ cation that is sandwiched between the outer benzene rings of o-terphenyl apparently stabilizes the $[M+Ag]^+$ cation.

3.3.5 Five- and Seven-Ringed PAH Compounds

To determine the efficiency of the Ag^+ cation interaction with the central ring, five- and seven-ringed PAH compounds were studied. With large ringed PAH compounds the π -electron conjugation increases. As previous studies have illustrated, however, the geometry of the PAH compound dictates the production of the $[\text{M}+\text{Ag}]^+$ cation. The addition of a benzene ring can either localize the π -electron density or delocalize it. This concept is illustrated with the following chosen PAH compounds.

Benzo[e]pyrene consists of five aromatic rings. The delocalized π electrons from the outer benzene rings contribute electron density to the central benzene ring A to provide a binding site for the Ag^+ cation. The 532-nm and 355-nm LDI/LA-TOFMS spectra of this PAH are shown in Figure 3.19. In both cases, a strong M^+ cation signal is observed accompanied with a weaker $[\text{M}+\text{Ag}]^+$ cation signal.

Benzo[a]pyrene is another five-ringed PAH compound. However, the fifth benzene ring H does not contribute its electron density to the central ring A. Consequently, the electron density is spread over the molecule instead of one central benzene ring. The 532-nm and 355-nm LDI/LA-TOFMS spectra of this PAH are illustrated in Figure 3.20. The M^+ signal was observed, but the $[\text{M}+\text{Ag}]^+$ cation was not produced using both wavelengths.

Perylene is another five-ringed PAH compound, whose structure is such that the four outer benzene rings contribute electron density to the central ring. The M^+ signal was observed only in the 355-nm LDI/LA-TOFMS spectrum, as illustrated in Figure 3.21.

Figure 3.19 LDI/LA-TOFMS spectra of benzo[e]pyrene ($m/z = 252$) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

Figure 3.20 LDI/LA-TOFMS spectra of benzo[a]pyrene (m/z 252) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

Figure 3.21 LDI/LA-TOFMS spectrum of perylene ($m/z = 252$) using a 355-nm laser beam

The last PAH compound studied was coronene. Its structure consists of six benzene rings surrounding a central benzene ring. Previous laser ablation experiments performed with a 266-nm wavelength did not fragment this molecule (Joblin et al., 1997). Furthermore, coronene reacted weakly with the Ag⁺ cation in the gas-phase to form a [M+Ag]⁺ cation when a growth channel region was used to promote gas-phase reactions (Buchanan et al., 1999). This reaction might not be expected to occur with our LDI/LA technique. The 532-nm and 355-nm LDI/LA-TOFMS spectra of this PAH are illustrated in Figure 3.22. The M⁺ signal was again observed using both wavelengths. However, a [M+Ag]⁺ cation was not observed in either mass spectrum although most of the electron density was thought to reside on the central benzene ring A.

Figure 3.22 LDI/LA-TOFMS spectra of coronene ($m/z \approx 300$) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

Apparently, the location of outer benzene rings plays a crucial role in determining the gas-phase ion chemistry. The fifth benzene ring F of benzo[e]pyrene causes the electron density to localize on the central benzene ring A, while the fifth benzene ring H of benzo[a]pyrene causes the electron density to delocalize and spread over the benzo[a]pyrene molecule. Both perylene and coronene have their outer benzene rings contribute electron density to a central benzene ring A. The $[M+Ag]^+$ cation was expected, in accordance with benzo[e]pyrene, but was not observed. This is further explained in Section 3.3.6 by their ionization energies relative to that of Ag.

3.3.6 Ionization Energy Dependency of the Cation- π Interaction

The appearance of M^+ and $[M+Ag]^+$ peaks in the LDI/LA-TOFMS spectra for PAH compounds can only be attributed to structural differences. These, of course, dictate the physical and chemical properties of the PAH compounds. The molar absorptivities of these PAH compounds are listed in Table 3.7. It is interesting to note that two PAH compounds that do not absorb the laser light do not necessarily produce similar mass spectra. For instance, perylene does not absorb the 532-nm wavelength and did not produce a 532-nm LDI/LA-TOFMS spectrum. In contrast, benzo[a]pyrene does not absorb the 532-nm wavelength and yet it produced a 532-nm LDI/LA-TOFMS spectrum with the $[M+Ag]^+$ cation. Multi-photon ionization does not appear to be responsible.

Table 3.7 Molar absorptivities ($M^{-1} \text{ cm}^{-1}$) of PAH compounds.

Compound	Molar absorptivity for a 532-nm wavelength	Molar absorptivity for a 355-nm wavelength
1,2-Benz[a]anthracene	0	10 ± 2
Benzo[a]pyrene	0	40 ± 2
Benzo[e]pyrene	0	2 ± 2
Chrysene	0	0
Coronene	0	0
Perylene	0	10 ± 2
Pyrene	0	0
Triphenylene	0	0

Hence, it is proposed that the formation of the $[M+Ag]^+$ cation, as described in Section 4.2.2, is the result of the gas-phase reaction:

When the $[M+Ag]^+$ cation is formed, the energy released is transferred to another species X^* within the ablation plume via gas-phase ion-molecule reactions. The Ag^+ cation- π bond, formed by Equation 3.3.6-1, is strong (Deng and Kebarle, 1998) and may be influenced by substituted functional groups. For instance, substitution of a hydrogen atom in the para position of the phenyl ring on poly(styrene) by a chlorine atom decreases the π electron density in the phenyl ring (Pleul et al., 1997). Similarly, the oxygen on anthrone, 9,10-anthroquinone, and 9,10-phenanthroquinone affects the π -electron density of the central ring. Clearly binding of the silver cation to the π face of an aromatic molecule would include contributions from charge-dipole, charge-induced dipole, charge-quadrupole, etc. intermolecular forces (Kim et al., 1994 A). It was even suggested that the interaction was predominantly charge-quadrupole (Williams, 1993). The Ag^+ cation- π interaction for nine PAH compounds was previously reported, although the complex structures were formed by ESI (Ng et al., 1998).

Ng et al. determined the chemical reactivity of the Ag^+ cation for PAH compounds by using dissociation rate constants (Ng et al., 1998). In this work, the silver cation affinity of PAH compounds can be explained by considering their ionization energies. PAH compounds with ionization energies higher than that of Ag form the cation- π bond in accordance with Equation 3.3.6-1. However, as the ionization energy of the PAH compound increases, the cation- π bond will become weaker. If the ionization

energy of the PAH compound is too high, the strength of the cation- π bond becomes so weak as to not form a bond at all. On the other hand, if the ionization energy of the PAH compound is lower than that of Ag, the cation- π bond becomes so weak that the silver may withdraw an electron from the PAH molecule:

This is an electron-transfer reaction that would proceed by either electron tunneling or some other pathways. The excess energy is transferred via gas-phase ion-molecule reactions to another species X within the ablation plume. Simple charge-transfer reactions require the ionization energies of the metal and the analyte to be close. $IE(\text{metal}) \geq IE(\text{analyte})$ (Dance et al., 1996). It has been suggested that the high π electron density of a benzene ring may be donated into the d_{xz} and d_{yz} orbitals of a silver atom, which shows electron acceptor properties. The Ag^+ cation increases the charge-transfer reaction due to its higher electron acceptor strength (Pleul et al., 1997).

To confirm Equation 3.3.6-2, the Ag^+ cation affinity difference between the 355-nm and 532-nm LDI/LA-TOFMS spectra must be discerned. This is further described in Section 4.2.2. Briefly, neutral Ag atoms produced by the 355-nm wavelength have an internal energy greater than those produced by the 532-nm wavelength. These neutral Ag atoms can transfer their internal energy to PAH molecules via gas-phase collisions. Consequently, PAH molecules with a higher internal energy will react with the Ag^+ cation. In contrast, the 532-nm wavelength only produces neutral Ag atoms with a low internal energy.

The 355-nm LDI/LA-TOFMS spectrum of each PAH compound was analyzed first. As shown in Equation 3.3.6-2, the summation of the M^+ and $[M+Ag]^+$ cation signal intensities (normalized to the Ag^+ cation signal intensity) is the total amount of $[M+Ag]^+$ cations formed before the reaction proceeds. This sum value can be used as an indicator of the Ag^+ cation affinity for each PAH. Table 3.8 lists the sum of the M^+ and $[M+Ag]^+$ cation signal intensities for the PAH compounds. Notice that anthracene and phenanthrene are not included on this list because they were thought to sublime under our vacuum conditions. Figure 3.23 illustrates the dependence of the M^+ and $[M+Ag]^+$ cation signal intensities (normalized to the Ag^+ cation signal intensity) on the ionization energy for the PAH compounds. It is evident that an ionization energy close to and above silver's ionization energy (7.58 eV) exhibits the $[M+Ag]^+$ cation. However, the M^+ cation signal intensity fluctuates. These fluctuations in the M^+ cation signal intensity are difficult to treat and a different indication of the silver cation affinity toward PAH compounds must be set up.

Considering that every M^+ cation observed in the 355-nm LDI/LA-TOFMS spectrum was produced from $[M+Ag]^+$ via Equation 3.3.6-2, the ratios $[M+Ag]^+/(M^+[M+Ag]^+)$ and $M^+/(M^+[M+Ag]^+)$ were calculated for each PAH compound. Table 3.9 lists the ionization energy and ratios for all of the PAH compounds. Figure 3.24 illustrates the ratios as a function of the ionization energy for the PAH compounds. Clearly, as the ionization energy of the PAH compound increases, the M^+ ratio decreases while the $[M+Ag]^+$ ratio increases, especially near silver's ionization energy of 7.58 eV. These results support the reaction given by Equation 3.3.6-2.

Table 3.8 The sum of M^+ and $[M+Ag]^+$ cation signal intensities (normalized to the Ag^+ cation signal intensity) for PAH compounds from a 355-nm laser beam.

Compound	$M^+ + [M+Ag]^+$ (V)
Pyrene	0.00 ± 0.00
Perylene	0.03 ± 0.02
Coronene	0.08 ± 0.04
Triphenylene	0.24 ± 0.06
Chrysene	0.45 ± 0.05
Benzo[e]pyrene	2.35 ± 0.16
1,2-Benz[a]anthracene	2.67 ± 1.76
Benzo[a]pyrene	4.59 ± 2.31

Figure 3.23 M^+ and $[M+Ag]^+$ cation signal intensities (normalized to the Ag^+ cation signal intensity) as a function of the ionization energy for PAH compounds

Table 3.9 Ionization energy (eV) and ratios for PAH compounds from a 355-nm laser beam.

Compound	IE (eV)	$\frac{[M+Ag]^+}{(M^+[M+Ag]^+)}$	$\frac{M^+}{(M^+[M+Ag]^+)}$
Perylene	6.96	0.00	100.00
Benzo[a]pyrene	7.10	0.00	100.00
Coronene	7.29	0.00	100.00
Benzo[e]pyrene	7.41	4.18	95.82
Pyrene	7.43	0.00	100.00
1,2-Benz[a]anthracene	7.45	5.54	94.45
Chrysene	7.60	28.82	71.18
Triphenylene	7.80	40.91	59.08

Figure 3.24 % Completion as a function of the ionization energies for PAH compounds using the 355-nm wavelength

A comparison between our LDI/LA method and the ESI by Ng et al. can be accomplished by measuring the relative Ag^+ cation affinity toward pyrene, triphenylene, chrysene and benz[a]anthracene. Ng et al. previously reported how the relative Ag^+ cation affinity toward these PAH compounds increased from pyrene to benz[a]anthracene, although it decreased for PAHs possessing a large number of bay regions (Ng et al., 1998). The trend observed using our LDI/LA technique is in excellent agreement with that of Ng et al. From our analysis, the PAH compounds with high ionization energies have their electron cloud significantly raised to promote π interactions with the Ag^+ cation. However, the PAH compounds that have low ionization energies promote the reaction given by Equation 3.3.6-2.

As mentioned above, the 532-nm LDI/LA-TOFMS results reveal the Ag^+ cation affinity toward some PAH compounds. Only 1,2-benz[a]anthracene, benzo[e]pyrene, benzo[a]pyrene and coronene react favorably with the Ag^+ cation when they do not have a high internal energy. Among these PAH compounds, only benzo[e]pyrene demonstrated the reaction given by Equation 3.3.6-2. Notice that three of these four PAH compounds have the largest Ag^+ cation affinity under 355-nm LDI/LA conditions, as listed in Table 3.8. Table 3.10 lists the Ag^+ cation affinity in increasing order for these four PAH compounds under 532-nm LDI/LA conditions. Notice that the Ag^+ cation affinity of benzo[a]pyrene under 532-nm LDI/LA conditions is smaller than that of benzo[a]pyrene with a larger internal energy under 355-nm LDI/LA conditions. Clearly the larger internal energy of a PAH compound generally enhances its Ag^+ cation affinity.

The Ag^+ cation- π interaction strength calculated by Ng et al. via a charge-quadruple model weakens when the aromatic π electrons are spread over the larger

Table 3.10 Increasing order of the sum of M^+ and $[M+Ag]^+$ cation signal intensities (normalized to the Ag^+ cation signal intensity) for four PAH compounds from a 532-nm laser beam.

Compound	$M^+[M+Ag]^+$ (V)
Benzo[a]pyrene	0.02
Coronene	0.08
Benzo[e]pyrene	0.08
1,2-Benz[a]anthracene	0.15

surface area of four or more fused aromatic rings (Ng et al., 1998). They demonstrated how the localization of the aromatic π electrons, depending on electron withdrawing/donating groups, and the structure of a PAH compound can enhance the Ag^+ cation affinity. From our analysis, the ionization energies of large PAH compounds were generally lower than small PAH compounds, and the aromatic π electrons are distributed throughout the molecule. We have established that the reactions given by Equations 3.3.6-1 and 3.3.6-2 occur. Clearly the Ag^+ cation- π bond formed by the reaction given by Equation 3.3.6-1 must be sufficiently weak and still provide the opportunity for the electron transfer reaction to occur. The strength of the Ag^+ cation- π bond is further tested using fullerenes, as described next in Section 3.3.7.

3.3.7 Fullerenes and C60 Derivative Compounds

The Ag^- cationization and electron-transfer reaction were further investigated with fullerene compounds ($\text{MW} > 720 \text{ Da}$), which are commonly known as unsaturated carbon buckminster balls. Since the ionization energy of fullerene compounds ($\sim 7.6 \text{ eV}$) is close to that of the silver atom, both the 355-nm and 532-nm LDI/LA-TOFMS spectra should exhibit a M^- and/or $[\text{M}+\text{Ag}]^-$ peak. Any M^- cation observed in the LDI/LA-TOFMS should be generated via Equations 3.3.6-1 and 3.3.6-2. Since the aromatic π -electrons are distributed evenly throughout the ball structure, a weak Ag^- cation- π interaction is expected (Ng et al., 1998), and the M^- cation should be more abundant than the $[\text{M}+\text{Ag}]^-$ cation. Lastly, sublimation of the sample should not occur when the molecular weight of each fullerene compound is very high ($>720 \text{ Da}$). It was previously reported that fullerenes exhibited M^- , $[\text{M}+\text{La}]^-$, $[\text{M}+\text{Gd}]^-$ and $[\text{M}+\text{Lu}]^-$ cation peaks via LA-TOFMS (Hopwood et al., 1997; Moro et al., 1993). As the ionization energies of lanthanum (5.58 eV), gadolinium (6.15 eV) and lutetium (5.43 eV) are smaller than those of the fullerenes, the electron transfer reaction in Equation 3.3.6-2 is not expected. Another report indicated single and double electron transfer reactions between triply charged fullerene C_{60}^{3-} and anthracene, corannulene and pyrene (Jahavery et al., 1993). Jahavery et al. justified that the PAH compounds transferred an electron to the triply charged fullerene using the ionization energy model. The cationic products were singly and doubly charge PAH compounds.

The 532-nm and 355-nm LDI/LA-TOFMS spectra of C60 fullerene ($m/z 720$) exhibited the M^- peak, as illustrated in Figure 3.25. The M^- peak was also observed in

Figure 3.25 LDI/LA-TOFMS spectra of C₆₀ (*m/z* 720) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

Figure 3.26 LDI/LA-TOFMS spectra of C70 ($m \approx 840$) using (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

Table 3.11 M^+ cation signal intensities (normalized to the Ag^+ cation signal intensity) for C60 and C70 fullerenes from a 532-nm and 355-nm laser beam.

Compound	M^+ (V) from a 532-nm laser beam	M^+ (V) from a 355-nm laser beam
C60	0.06	0.07
C70	0.04	0.03

the 532-nm and 355-nm LDI/LA-TOFMS spectra of C70 fullerene (m/z 840), as illustrated in Figure 3.26. Neither fullerene exhibited the $[M+Ag]^+$ cation peak. The Ag^+ cation affinities (given as the M^+ cation signal intensity normalized to the Ag^+ cation signal intensity) of both compounds at both wavelengths are listed in Table 3.11. Comparison between the 532-nm and 355-nm LDI/LA-TOFMS spectra of C60 indicates that the Ag^+ cation affinities are approximately the same. The 532-nm and 355-nm LDI/LA-TOFMS spectra of C70 also indicate that the Ag^+ cation affinities are approximately the same. Clearly the π -electrons of both fullerenes have sufficiently strong interactions with the Ag^+ cation. Also, the fullerenes still react via Equations 3.3.6-1 and 3.3.6-2 to produce the M^+ cation. Furthermore, as the surface area of the fullerene molecule increases, the Ag^+ cation affinity decreases (the normalized M^+ cation signal intensity for C70 is approximately half that for C60). Previous reports observed coalescence products of C_n^+ ($n \geq 60$) (Beck et al., 1994). These were not observed in our LDI/LA-TOFMS spectra probably due to the high laser fluence requirement of 1000 mJ/cm² (Liu et al., 1995; Hunter et al., 1994), that was not attainable with our Nd-Hyper YAG laser (and focusing conditions).

Fullerene derivative compounds synthesized for inhibiting HIV enzymes had previously been characterized by MALDI-TOFMS (Friedman et al., 1998; Ballenweg et al., 1996; Sijbesma et al. 1993). Two fullerene derivative compounds that do not inhibit HIV enzymes, 1,2-(dimethoxymethano) fullerene C₆₀ (m/z 795) and tert-butyl (1,2-methanofullerene C₆₀)-61-carboxylate (m/z 835), were tested to investigate the Ag⁺ cation affinity and to evaluate our LDI/LA technique for characterizing HIV inhibitors. Both the 532-nm and 355-nm LDI/LA-TOFMS spectra of these compounds exhibited the C₆₀⁺ cation peak, as shown in Figure 3.27 and Figure 3.28. Also, in low abundance (labeled as an arrow in Figures 3.27 and 3.28), the 532-nm LDI/LA-TOFMS spectrum of 1,2-(dimethoxymethano) fullerene C₆₀ exhibited a peak at approximately m/z 757, while the 532-nm LDI/LA-TOFMS spectrum of tert-butyl (1,2-methanofullerene C₆₀)-61-carboxylate exhibited a peak at approximately m/z 767. Their appearance suggested that an intermediate reaction occurred between the reactions given by Equations 3.3.6-1 and 3.3.6-2, and was governed by the internal energy of the Ag⁺ cation. The Ag⁺ cation affinity (given as the sum of the C₆₀⁺ cation signal intensity and fragment signal intensity normalized to the Ag⁺ cation signal intensity) of both compounds at both wavelengths are listed in Table 3.12. Previous reports by Ong et al illustrated the coalescence product in a LDI-TOFMS spectrum using a low laser fluence of 8 mJ/cm² (Ong et al., 1997). Notice that the Ag⁺ cation affinity of tert-butyl (1,2-methanofullerene C₆₀)-61-carboxylate is greater when it has a higher internal energy under 355-nm LDI/LA conditions. Also notice that the C₆₀⁺ cation signal intensities at both wavelengths from tert-butyl (1,2-methanofullerene C₆₀)-61-carboxylate are greater than those for 1,2-(dimethoxymethano) fullerene C₆₀. This indicates that the Ag⁺ cation affinity for tert-

Table 3.12 Sum of C_{60}^+ and fragment cation signal intensities (normalized to the Ag^+ cation signal intensity) for C_{60} derivative compounds from a 532-nm and 355-nm laser beam.

Compound	Cation signal intensity (V) from a 532-nm laser beam	Cation signal intensity (V) from a 355-nm laser beam
1,2-(Dimethoxymethano) fullerene C_{60}	0.010	0.010
Tert-butyl (1,2-methanofullerene C_{60})-61-carboxylate	0.013	0.048

butyl (1,2-methanofullerene C_{60})-61-carboxylate is greater than that for 1,2-(dimethoxymethano) fullerene C_{60} . This may be attributed to the additional carboxylate oxygen binding site for the Ag^+ cation. The dipole interaction from the oxygen on the carboxylate from tert-butyl (1,2-methanofullerene C_{60})-61-carboxylate attracts the Ag^+ cation more than the 1,2-(dimethoxymethano) fullerene C_{60} .

The Ag^+ cation affinity of fullerenes and fullerene derivative compounds are generally smaller than that of PAH compounds, as demonstrated by the smaller cation signal intensities (normalized to the Ag^+ cation signal intensity). Even though the ionization energies of the fullerene compounds are similar to that of silver, the $[M+Ag]^+$ cation dissociates to the more stable M^+ cation form. An intermediate reaction appears to occur when using a lower internal energy of Ag^+ cation. The cation- π interaction between Ag^+ and the two fullerenes is still sufficiently strong, but appears to be weaker for the two fullerene derivatives. However, the Ag^+ cation affinity toward the carboxylate functional group in tert-butyl (1,2-methanofullerene C_{60})-61-carboxylate appears to be greater than that for the alcohol functional group in 1,2-

(dimethoxymethano) fullerene C₆₀. Hence, this technique could potentially be applied to characterize biologically significant compounds.

Figure 3.27 LDI/LA-TOFMS spectra of 1,2-(dimethoxymethano)fullerene C₆₀ (m/z 795) using: (A) 532-nm laser beam, and (B) a 355-nm laser beam.

Figure 3.28 LDI/LA-TOFMS spectra of tert-butyl (1,2-methanofullerene C₆₀)-61-carboxylate (m/z 835) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

3.4 Thia Fatty Esters and Acids

3.4.1 Experimental Details

Gas-phase ion/molecule reactions with charge-remote fragmentation were evaluated with our LDI/LA technique on thia fatty esters and acids. Front-illumination LDI/LA-TOFMS spectra of thia fatty esters and acids were obtained using the fifth configuration (refer to Figure 2.14 C). The parameters set on the LDI/LA-TOFMS instrument are listed in Table 3.13. Notice that the voltages for grid A and grid B were changed to optimize the resolution of the LDI/LA-TOFMS. A single-shot mass spectrum was recorded, and peaks that had a signal-to-noise ratio greater than 5:1 were recognized.

Table 3.13 Operational settings of the LDI/LA-TOFMS instrument.

Parameter	Value
Pressure P (torr)	$0.3\text{--}1.5 \times 10^{-6}$
Laser wavelengths (nm)	355 and 532
Laser fluence (mJ/cm^2)	Minimum
Laser repetition rate (Hz)	1
Laser pulse width at FWHM (ns)	8
Lens focal length (cm)	50
Drift tube length D (cm)	42.2
Length s (cm)	0.4
Length d (cm)	2.1
Grid a voltage (kV)	10
Grid b voltage (kV)	9.6
Grid c voltage (kV)	0
Front-face voltage on MCP (kV)	-1.6

Table 3.14 (Thia) fatty esters and acids, and their molecular weights.

Compound	MW
Methyl 5-oxooctadecanoate	312
Methyl 9-thiaheptadecanoate S-oxide	318
Methyl 9-thiaheptadecanoate S-dioxide	334
Methyl 9-thiaheptadecanoate	302
Methyl heptadecanoate	284
9-Thiaheptadecanoic acid	288
3-Thiatetradecanoic acid	246

Methyl 5-oxooctadecanoate was purchased from Aldrich (Milwaukee, WI). All other thia fatty esters and acids were synthesized by Buist et. al., according to standard procedures and were well characterized by IR, MS and NMR. Their names and molecular weights are listed in Table 3.14, and their molecular structures are illustrated in Figure 3.29.

Sample solutions at a concentration of 10 mg/ml were prepared by dissolving a (thia) fatty acid or ester in chloroform (HPLC grade). A 50- μ l aliquot of each sample solution was allowed to dry on a thin silver film substrate (2.5 cm x 2.5 cm) in air. The dry sample overlayer on the substrate was placed in the ion source chamber of the TOFMS instrument for LDI/LA analysis.

Figure 3.29 Molecular structures of (thia) fatty esters and acids.

3.4.2 Silver Cationization to Carboxyls and Sulfoxides

Reaction pathways for both fatty acids and oxofatty acids cationized by Li^+ and other alkali metal ions were previously examined via collisionally activated dissociation (CAD) (Cordero and Wesdemiotis, 1994; Cheng and Gross, 1998). Even though those experiments indicated that larger alkali metals exhibited weaker metal-keto bond strength, transition metals might provide better selectivity for the cationization of fatty acids and esters with different functional groups in the long alkyl chain. The strength of silver-keto bonds was studied by the present LDI-TOFMS technique using methyl 5-oxooctadecanoate (m/z 312) as a model oxofatty ester. Its LDI/LA-TOFMS spectrum using a 532-nm laser beam is shown in Figure 3.30 A. Minor mass spectral peaks corresponding to impurities are thought to originate from the thin silver substrate and are observed at low signal intensity. Major mass spectral peaks from the sample substrate are seen at m/z 23, 40, 108, 216 and 323, in addition to the silver-cationized analyte molecule, $[\text{M}+\text{Ag}]^+$, at m/z 420. Similar results were observed in the LDI-TOFMS spectrum of methyl 5-oxooctadecanoate using a 355-nm wavelength, as shown in Figure 3.30 B. Clearly silver has a good affinity towards either the keto functional group or the carboxyl group in this oxofatty ester, regardless of the wavelength used for LDI. It is important to note that this fatty ester was not sodiated or calciated despite the great abundance of Na^+ and Ca^+ in the ablation plume, even though oxofatty esters are known to exhibit lithiation. Despite its large cation size, Ag^+ appears to cationize methyl 5-oxooctadecanoate effectively and demonstrates a strong enough interaction to maintain

Figure 3.30 LDI/LA-TOFMS spectra of methyl 5-oxooctadecanoate (m/z 312) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

its structure as the $[M+Ag]^+$ cation. In fact, the Ag^+ appears to possess a greater chemical reactivity in the gas-phase toward the oxofatty ester than Na^+ and Ca^+ .

Lithiation, sodiation and calciation experiments performed on oxofatty acids indicated that charge-remote fragmentation (CRF) occurred and that a fixed charged site was essential for the formation of CRF product ions via a pericyclic 1,4-hydrogen elimination rearrangement (Cheng et al., 1998 A) or a simple homolytic cleavage (Cheng et al., 1998 B). Since there does not appear to be any major CRF product signal from the methyl 5-oxooctadecanoate, CAD might be required for their abundant generation. To test this hypothesis, the effect of a different functional group along the alkyl chain was evaluated with a sulfoxide. Using conventional electron impact (EI) mass spectrometry, the fragmentation pattern of methyl 9-thiaheptadecanoate S-oxide (m/z 318) indicated cleavage as evidenced by the expected $[SO(CH_2)_yCH_3]^+$ and $[CH_3OCO(CH_2)_x]^+$ (where $x \leq 7$ and $y \leq 7$) products, but no CRF products were observed. This fragmentation pattern was identical to the fragmentation pattern reported previously (Christie et al., 1991). When this compound was analyzed by the present LDI/LA-TOFMS technique using a 532-nm laser beam, the spectrum shown in Figure 3.31 A was obtained. Substrate mass spectral peaks are seen at m/z 23, 40, 108, 216 and 323. More importantly, the silver-cationized analyte $[M+Ag]^+$ is observed at m/z 426. Since the carboxyl oxygen and the sulfoxide oxygen are both electron-rich sites, it is impossible to determine where the silver ion resides.

The LDI/LA-TOFMS spectrum of methyl 9-thiaheptadecanoate S-oxide obtained using a 355-nm laser beam is shown in Figure 3.31 B, which exhibits substrate mass spectral peaks at m/z 23, 108, 216 and 323. In addition, a large silver-cationized peak,

$[M+Ag]^+$, is seen at m/z 426. A conglomerate of fragments is also observed in the range from m/z 265 to m/z 355. Since there is no appreciable absorption of either 355-nm or 532-nm wavelength by the analyte molecule, we hypothesize that a different fragmentation mechanism for the 355-nm wavelength resulted from more energetic electronic excitation of the thin silver film substrate during the LDI/LA process. Proposed structures of the conglomerate of fragments based on CRF patterns are shown in Figure 3.32. Each dotted line in Figure 3.32 A points to two related structures where the silver cation is found at different locations. These many structures can undergo additional rearrangement reactions, where the methyl terminal bond of the ester cleaves and a CH_2 unit may be added to the alkyl chain (not shown in Figure 3.32 A). Lastly, the structures shown in Figure 3.32 B correspond to fragments that can only be formed by attachment of the silver cation to the sulfoxide. The oxygen on the S-oxide has a high electronegativity, permitting silver cation attachment at this location as well as the oxygen at the carboxyl location. Consequently, a conglomerate of fragments is observed in the 355-nm mass spectrum. These structures are seen with similar abundances in the spectrum, suggesting equivalent silver cation affinity between $S=O$ and $C=O$. It has recently been reported that Ag^+ is preferentially chelated by two functional groups in the gas phase (Deng and Kebarle, 1998).

Figure 3.31 LDI/LA-TOFMS spectra of methyl 9-thiaheptadecanoate S-oxide (m/z 318) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

Figure 3.32 Formation of CRF products near the sulfoxide group through silver cationization of methyl 9-thiaheptadecanoate S-oxide (m/z 318). (A) Solid line points to a structure with the silver cation located at the carboxyl group and each dotted line points to two related structures with the silver cation located at either the carboxyl or sulfoxide group. (B) Structures with the silver cation located at the sulfoxide group.

3.4.3 Silver Cationization to Sulfur-dioxide and Sulfur-ether

The EI mass spectrum of methyl 9-thiaheptadecanoate S-dioxide (m/z 334) exhibited a fragmentation pattern similar to the one for methyl 9-thiaheptadecanoate S-oxide. Upon analyzing this S-dioxide by the present LDI/LA-TOFMS technique, substrate mass spectral peaks at m/z 23, 40, 108, 216, and 323 were again observed in the 532-nm spectrum, as shown in Figure 3.33 A. The silver-cationized analyte $[M+Ag]^+$ was observed at m/z 442. A peak was found at m/z 356, which was either a γ fragment or $[M+Na]^+$ at m/z 357. Next, the LDI/LA-TOFMS spectrum of methyl 9-thiaheptadecanoate S-dioxide obtained using a 355-nm wavelength is shown in Figure 3.33 B, in which the substrate mass spectral peaks were seen at m/z 23, 40, 108, 216 and 323. The silver cationized analyte $[M+Ag]^+$ and a β fragment were observed at m/z 442 and m/z 250, respectively. A comparison between Figure 3.31 and Figure 3.33 shows that different CRF products were observed for the two oxidation states of sulfur in the S-oxide and S-dioxide functional groups. The fragment structures would be analogous to those proposed in Figure 3.32.

Thia fatty esters are sulfur derivatives of fatty esters in which one of the methylene groups in the fatty ester chain is replaced by a sulfur atom (Buist and Dimnik, 1986). The EI mass spectrum of methyl 9-thiaheptadecanoate (m/z 302) indicated characteristic $[S(CH_2)_yCH_3]^+$ and $[CH_3OCO(CH_2)_x]^+$ (where $x \leq 7$ and $y \leq 7$) fragments, but not CRF products. The 532-nm spectrum of this thia fatty ester analyzed by the present LDI-TOFMS technique is shown in Figure 3.34 A. The substrate mass spectral peaks at m/z 23, 40, 108, 216 and 323 were again observed. Surprisingly no silver-cationized

Figure 3.33 LD/LA-TOFMS spectra of methyl 9-thiaheptadecanoate S-dioxide (m/z 334) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

peak $[M+Ag]^-$ was found at m/z 410. This clearly indicates that the presence of oxygen on the sulfur is essential for silver cationization to form stable adducts, although no implication is made here to restrict silver cationization to the sulfur oxygen(s). In addition, a β fragment $[CH_3COOAg(CH_2)_4CHCH_2]^-$ at m/z 250 was observed. This β fragment at m/z 250 was thought to have the same structure as that observed for methyl 9-thiaheptadecanoate S-dioxide. The formation of CRF products appears to be dictated by the presence or absence of oxygen on the sulfur and implies alternative fragmentation processes. This behavior is in stark contrast to the case of oxofatty acids, where both β and γ fragments are usually formed if the oxo group is located at least 4 C-C bonds from the carboxylate and 3 C-C bonds from the hydrocarbon end (Cheng et al., 1998 A). Furthermore, Figure 3.34 B shows the 355-nm LDI-TOFMS spectrum of methyl 9-thiaheptadecanoate. Substrate mass spectral peaks were again observed, but there did not appear to be any silver-cationized analyte or fragmentation peaks. The absence of $[M+Ag]^-$ suggested that the molecular ion dissociated completely under these LDI/LA conditions, even if it was initially formed.

Figure 3.34 LDI/LA-TOFMS spectra of methyl 9-thiaheptadecanoate ($m \approx 302$) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

3.4.4 Different Fragmentation Mechanisms for Fatty Esters

Silver ions in the gas-phase are known to be reactive towards a variety of organic compounds such as hydrocarbons and carbonyls (Eller and Schwarz, 1991), in addition to thia fatty esters. For comparison purposes, the LDI/LA-TOFMS spectrum of methyl heptadecanoate (m/z 284) was obtained using a 532-nm wavelength. As shown in Figure 3.35 A, it exhibited a silver-cationized analyte at m/z 392 and the substrate mass spectral peaks. The fact that silver cationization occurs with methyl heptadecanoate but not methyl 9-thiaheptadecanoate demonstrates the stronger affinity of Ag^+ for the non-thia fatty ester. It may even suggest that the presence of sulfur in the thia fatty ester impedes silver cationization.

The LDI/LA-TOFMS spectrum of methyl heptadecanoate obtained using a 355-nm wavelength is shown in Figure 3.35 B. The substrate mass spectral peaks are observed, as well as the silver-cationized analyte at m/z 392. Interestingly a fragmentation of large abundance appears at m/z 377, corresponding probably to either $[\text{COOAg}(\text{CH}_2)_{15}\text{CH}_3]^-$ or $[\text{AgCH}_3\text{OOC}(\text{CH}_2)_{15}]^-$. The former represents the loss of a methyl group from the carboxylate terminal of the silver-cationized analyte via charge-proximal fragmentation, while the latter represents the loss of a methyl group at the terminating end of the alkyl chain via CRF. Without labeling, an unequivocal answer cannot be given. Previous EI mass spectrometry performed on methyl stearate, a structurally similar compound, indicated that larger total ion yields would result from larger electron impact energies (Takayama et al., 1995). Analogous behavior may have

Figure 3.35 LDI/LA-TOFMS spectra of methyl heptadecanoate (m/z 284) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

occurred under our experimental conditions where the 1.4 eV difference between the 355-nm (3.7 eV) and the 532-nm (2.3 eV) wavelengths was significant. The 355-nm wavelength should have a larger photon energy for the fragmentation of the silver-cationized analyte.

3.4.5 Silver Cationization of Thia Fatty Acids

Since only alkali metal cationization of non-thia fatty acids were previously reported (Madhusudanan, 1995), silver cationization of thia fatty acids was investigated. The LDI/LA-TOFMS spectrum obtained using a 532-nm wavelength on a 9-thiaheptadecanoic acid (m/z 288) is shown in Figure 3.36 A. The substrate mass spectral peaks are observed, but none of the silver-cationized analyte, β fragments and γ fragments can be seen. Similarly, the corresponding 355-nm LDI/LA-TOFMS spectrum shown in Figure 3.36 B reveals the same behavior. The silver ion reactivity available in the present LDI/LA-TOFMS technique can make a distinction between thia fatty esters and acids.

The LDI/LA-TOFMS spectra of 3-thiatetradecanoic acid (m/z 246) using a 532-nm and a 355-nm wavelength do not exhibit the silver-cationized analyte, β fragments or γ fragments, as shown in Figures 3.37. The position of the sulfur atom in the alkyl chain does not seem to affect the silver affinity towards these two thia fatty acids. Apparently, the terminal functional group (H vs. CH_3) plays an important role in determining the outcome of silver cation attachment to the thia fatty acids versus esters. Differences in dipole moment and polarizability between thia fatty acids and esters may explain the absence of a $[\text{M}+\text{Ag}]^+$ peak for the former (Guo et al., 1989). Moreover, the presence of the sulfur atom in the alkyl chain hinders the Na^+ and Ca^+ chemical reactivity because $[\text{M}+\text{Na}]^+$ and $[\text{M}+\text{Ca}]^+$ was not observed using this LDI/LA technique.

Figure 3.36 LDI/LA-TOFMS spectra of 9-thiaheptadecanoic acid ($m = 288$) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

Figure 3.37 LDI/LA-TOFMS spectra of 3-thiatetradecanoic acid (m/z 246) using: (A) a 532-nm laser beam, and (B) a 355-nm laser beam.

Chapter 4: LDI/LA-TOFMS Physical Processes

4.1 Different Silver Film Thicknesses

4.1.1 Experimental Details

The LDI/LA technique was next evaluated for quantitative analysis. Single-shot mass spectra were first analyzed to confirm that sample peaks were not observed randomly. Back-illumination LDI/LA-TOFMS spectra of a thin silver film and gramicidin S deposited on a thin silver film were obtained using the third configuration (refer to Figure 2.9 A). The parameters set on the LDI/LA-TOFMS instrument are listed in Table 4.1. Over 50 mass spectra were summed and averaged to evaluate internal standardization. A peak that had a signal-to-noise ratio greater than 5:1 was recognized as a peak.

Table 4.1. Operational settings of the LDI/LA-TOFMS instrument.

Parameter	Value
Pressure P (torr)	1.5×10^{-6}
Laser wavelength (nm)	532
Laser fluence (mJ/cm^2)	14-250
Laser repetition rate (Hz)	1
Laser pulse width at FWHM (ns)	8
Lens focal length (cm)	25
Drift tube length D (cm)	42.2
Length s (cm)	1.0
Length d (cm)	-
Grid a voltage (kV)	20
Grid b voltage (kV)	0
Grid c voltage (kV)	0
Front-face voltage on MCP (kV)	-1.6

A 10- μ L aliquot of 4 mM gramicidin S solution in methanol was applied to each 2.5 cm x 2.5 cm Lumonics or Tollen's thin silver film. After air drying, the sample was placed inside the ion source chamber of the LD/LA-TOFMS spectrometer for analysis. The thickness of the silver film was determined by measuring its spectrophotometric absorbance in a UV-VIS spectrophotometer (a glass microscope slide was the reference). Since the spectrophotometric absorbance of the Lumonics thin silver film was measured and the thickness of the Lumonics thin silver film was 40 nm, the thickness of the Tollen's thin silver films was approximated using Beer's Law.

4.1.2 Gramicidin S Signal and Laser Fluence Relationship

To determine the effectiveness of Ag^+ as a cationizing agent, different concentration ratios of silver to sample must be analyzed. These ratios were obtained by increasing the silver film thickness for a constant amount of sample. The sample chosen for these experiments was gramicidin S, a cyclic decapeptide demonstrated in Section 3.2.3 to react well with Ag^+ in gas-phase cationization. The $[\text{gramicidin S} + \text{Ag}]^+$ cation signal intensity attained a maximum value of 440 mV at an optimal silver film thickness (~ 30 nm) and an optimal incident laser energy of 140 $\mu\text{J}/\text{pulse}$. Furthermore, the $[\text{gramicidin S} + \text{Ag}]^+$ cation signal intensity was studied as a function of the transmitted laser energy through the Tollen's thin silver film or the absorbed laser energy by the Tollen's thin silver film. However, at a constant laser energy setting, the $[\text{gramicidin S} + \text{Ag}]^+$ cation signal intensity reproducibility was somewhat limited for a given Tollen's thin silver film thickness. The dependence of the average $[\text{gramicidin S} + \text{Ag}]^+$ cation signal intensity from all laser energies on Tollen's thin silver film thickness is illustrated in Figure 4.1. This graph shows a maximum $[\text{gramicidin S} + \text{Ag}]^+$ cation signal intensity at an optimal Tollen's thin silver film thickness. The vertical bars on the data points are an indication of the strong influence of different laser energies on the $[\text{gramicidin S} + \text{Ag}]^+$ cation signal intensity. Too thick and too thin silver films produce a low Ag^+ concentration during LDI/LA, which is most likely attributed to the LDI/LA mechanism, further described in Section 4.2.3. Indeed, this is analogous to the optimal analyte-to-matrix ratio requirement in MALDI.

Figure 4.1 A trend line showing dependence of the average [gramicidin S + Ag]⁺ cation signal intensity on the Tollen's thin silver film thickness (as represented by its spectrophotometric absorbance).

Another important realization is the effect of a different Tollen's thin silver film thickness on the laser beam incident on, transmitted through, and absorbed by the silver film. Firstly, there is an optimum incident laser energy for generating a maximum [gramicidin S + Ag]⁺ cation signal intensity for each Tollen's silver film thickness. Also, at these maximum [gramicidin S + Ag]⁺ cation signal intensities for different silver film thicknesses, the optimum incident laser energy appears to increase to a maximum value (from 70 to 175 $\mu\text{J/pulse}$) and then decrease (from 175 to 40 $\mu\text{J/pulse}$). Secondly, at these maximum [gramicidin S + Ag]⁺ cation signal intensities for different silver film thicknesses, the transmitted laser energy increases (from 50 to 70 $\mu\text{J/pulse}$) and then decreases (from 70 to 5 $\mu\text{J/pulse}$). Lastly, at the maximum [gramicidin S + Ag]⁺ cation signal intensities for different silver film thicknesses, the laser energy absorbed by the

silver film first increases (from 20 to 115 $\mu\text{J}/\text{pulse}$), and then decreases (from 115 to 35 $\mu\text{J}/\text{pulse}$). It is speculated that since thermal conductance varies with distance from the thin silver film backside to the film surface, more energy must be supplied on the backside of a thick silver film to desorb gramicidin S molecules off the film surface. It was reported that suitable combination of sample thickness and laser power density allowed the reproducible selection of a defined ionization mode (Lindner and Seydel, 1985). The LDI/LA mechanism is further investigated in Section 4.2.3.

4.1.3 Internal Standardization and Ion Association

The internal standard method is commonly used for quantitative analysis of unknown samples. Although LDI techniques do not use an internal standard usually, several papers have reported its use. For example, an internal standard was used in a new excitation scheme that could deposit higher amounts of internal energy in ions than conventional resonant excitation (Qin and Chait, 1996). It was also used to investigate the effect of repeat units in polymers on the cationization efficiency during the LDI of thin metal films (Cromwell et al., 1993). In our LDI/LA-TOFMS experiments, the Ag^+ cation originating from the Tollen's thin silver film was used as an internal standard to evaluate the effectiveness of gramicidin S sample cationization. Even though the silver film was used as an internal standard substance, it was also a source of Ag^+ cations for ionizing the sample molecules (Piccirillo et al., 1997). As described previously, a different film thickness would provide a different cationization efficiency for gramicidin S. One requirement for this internal standard method to work is that the Ag^+ cation can associate with the sample molecule, as described below.

Statistical analysis of particle emission from surfaces can provide a wealth of new or under-exploited information on particle production mechanisms. Among the statistical treatments of data, multiplicity describes the randomness of particle emission processes (da Silveira et al., 1998), while ion association describes the occurrence of two or more ions that are seen with high probability in the LDI/LA-TOFMS spectra (Huang et al., 1989). These two treatments can provide grounds for coincidence counting analysis.

Ion association is defined as the observation of two or more ions in the LDI/LA-

TOFMS spectra produced from the same sample. Figure 4.2 shows an ion association map (Schoer et al., 1990). A line drawn between two ions in the ion association map indicates association between them meaning that the ions are observed together with high probability in replicate LDI/LA-TOFMS spectra. Two ions that are not connected by a line are not associated, meaning that the ions are not observed together frequently in replicate LDI/LA-TOFMS spectra. The reasons for ion association in the present LDI/LA experiments are unknown, although it can be speculated that physical processes and chemical affinities play an important role. Also, the probability of observing the two ions can be dependent upon the laser photon energy. Three possible scenarios dictate what an ion association map will look like. Either two ions are efficiently ionized together, or two ions are not efficiently ionized together, or one of two ions is efficiently ionized.

Figure 4.2 The ion association maps of: (A) a Lumonics thin silver film, and (B) a gramicidin S sample deposited on a Lumonics thin silver film.

To determine the effectiveness of silver as a cationization reagent, ion association maps for the cations of interest were drawn. The ion association maps of a Lumonics thin silver film and a gramicidin S sample deposited on a Lumonics thin silver film are illustrated in Figure 4.2. Clearly, not all cations that were observed in each single-shot LDI/LA-TOFMS spectrum are associated. For example, the Ag^+ cation and the Ag_9^+ cation generated from a Lumonics thin silver film are not associated, meaning that the Ag_9^+ cation was not very frequently observed together with the Ag^+ cation. However, the $[\text{gramicidin S} + \text{Na}]^+$ and $[\text{gramicidin S} + \text{Ag}]^+$ cations generated from a gramicidin S sample deposited on a Lumonics thin silver film are associated with the internal standard Ag^+ cation. Clearly, the ion association maps illustrate the high degree of association or the high probability of observing the $[\text{gramicidin S} + \text{Na}]^+$ and the $[\text{gramicidin S} + \text{Ag}]^+$ cations together with the $[\text{Ag}_n]^+$ ($1 \leq n \leq 3$) cations in each LDI/LA-TOFMS spectrum.

Although ion association was developed to provide insight into ionization processes (Cotter, 1994), it had never been used with internal standard analysis. For example, in many of the presented results, the 532-nm and 355-nm LDI/LA-TOFMS mass spectra of samples differ considerably, which was largely attributed to the internal energy of the Ag^+ cation (hence cationization efficiency), further described in Section 4.2.2. In this study, however, ion association was performed to evaluate the Ag^+ cation affinity for gramicidin S. If the $[\text{gramicidin S} + \text{Ag}]^+$ and the Ag^+ cation signal intensities were measured from many LDI/LA-TOFMS spectra, they could be plotted against each other to obtain a $[\text{gramicidin S} + \text{Ag}]^+/\text{Ag}^+$ ratio. Clearly, both cations must be observed together with high probability in many LDI/LA-TOFMS spectra - they must be

Figure 4.3 Ion association and dissociation quadrants on a typical internal standard plot.

associated. Now we can define where ion association occurs on an internal standard plot, which consists of ion association and dissociation quadrants as illustrated in Figure 4.3. The x-axis and y-axis of this plot not only are the threshold limits for ion association and ion dissociation, but they also define where internal standardization occurs. The straight line represents the maximum sample signal intensity observed with a specific Ag^+ cation signal intensity when both of these ions are associated. Notice that this plot is created when both ions are associated under the assumption that the $[\text{gramicidin S} + \text{Ag}]^+$ and the Ag^+ cation signal intensities are independent of the laser energy imparted to the thin silver film. In fact, the laser fluence required for LA of thin silver films was constant for

thicknesses less than 60 μm (Andrew et al., 1983). The sign of the slope is an indication whether a positive or negative correlation exists between the internal standard and the sample cation when they are associated.

To determine the effectiveness of silver acting as a cationization reagent, an internal standard plot for a constant thin silver film thickness illustrated the relationships between the $[\text{gramicidin S} + \text{Na}]^+$ and $[\text{gramicidin S} + \text{Ag}]^+$ cation signals and the Ag^+ cation signal. These plots are shown in Figure 4.4 using the 532-nm wavelength. A linear relationship is only observed between the $[\text{gramicidin S} + \text{Ag}]^+$ cation and the Ag^+ cation, as illustrated in Figure 4.4 B. This linear relationship confirms the cationizing power of silver. When the internal standard plot for a different Tollen's thin silver film thickness was constructed, the same linear relationship was again observed. Recall that this linear relationship is independent of the laser fluence. Table 4.2 lists the slopes calculated from the linear plots for different Tollen's thin silver film thicknesses, as represented in spectrophotometric absorbance units.

Table 4.2 Table of the slopes ($\pm$ errors) for different thicknesses of Tollen's thin silver film.

Thickness (spectrophotometric absorption units)	Slope
0.10	0.27 ($\pm$ 0.02)
0.41	0.21 ($\pm$ 0.04)
0.63	0.12 ($\pm$ 0.05)
1.20	-0.01 ($\pm$ 0.06)
1.44	-0.04 ($\pm$ 0.05)

Figure 4.4 Dependence of: (A) $[\text{gramicidin S} + \text{Na}]^+$, and (B) $[\text{gramicidin S} + \text{Ag}]^+$ with the Ag^+ internal standard using the 532-nm wavelength.

The slope of each line indicates the $[\text{gramicidin S} + \text{Ag}]^+$ signal intensity with respect to the Ag^+ signal intensity, and it is the largest for the thinnest Tollen's thin silver film. The error on the slope is thought to be due to both the LDI/LA mechanism and the non-uniformity of the Tollen's thin silver film with the gramicidin S sample. Thin silver films primarily undergo electronic-excitation desorption, thicker silver films undergo thermal desorption, and very thick silver films do not desorb. Since electronic-excitation desorption of atoms and molecules occur preferentially from defects on surfaces (sites with low coordination numbers) (Dickinson et al., 1994; Hoheisel et al., 1993; Hattori et al., 1991; Vollmer and Trager, 1987; Nishi et al., 1984), it is not unreasonable to observe random $[\text{Ag}_n]^+$ cluster peak signal intensities using our LDI/LA technique. This is described in greater detail in Section 4.2.3.

To provide insight to the dependence of the amount of gramicidin S that was cationized on the Ag^+ concentration within the ablation plume, Figure 4.5 was constructed with the values from Table 4.2. It is a plot of the slopes (i.e. the $[\text{gramicidin S} + \text{Ag}]^+$ signal intensity versus the Ag^+ signal intensity) against the thicknesses of Tollen's thin silver films. This is identical to the analysis performed by Cromwell et al. where a cation complex signal was normalized to a Cu^+ signal (Cromwell et al., 1993). Although previous studies on MALDI had shown that the protonated analyte signal area was a non-linear function of the matrix-to-analyte concentration (Beeson et al., 1995), our results indicated a linear relationship. This was expected because Beeson et al. did not use an internal standard to measure the protonated analyte signal area. The negative slope of $-0.23 (\pm 0.02)$ indicates that the thicker silver films (hence a greater concentration of silver molecules and ions in the ablation plume) produce lower

Figure 4.5 Dependence of the $\Delta[\text{Gramicidin S} + \text{Ag}]^+ / \Delta \text{Ag}^+$ with the Tollen's thin silver film thickness (as represented by its spectrometric absorbance).

$[\text{gramicidin S} + \text{Ag}]^+$ cation signal intensities, while thinner silver films produce higher $[\text{gramicidin S} + \text{Ag}]^+$ cation signal intensities. Again, the 9 % error on the slope indicates that these results may be under the influence of the two discrepancies (mechanism and non-uniformity) mentioned earlier. Although the error bars on the data points are large, this plot qualitatively demonstrates the dependence of the $[\text{gramicidin S} + \text{Ag}]^+$ cation formation efficiency on the silver film thickness. Lastly, a comparison between the error bars of thick and thin silver films illustrates that the $[\text{M} + \text{Ag}]^+ / \text{Ag}^+$ ratio for thinner silver films is more reproducible than thicker silver films.

The maximum $[\text{gramicidin S} + \text{Ag}]^+$ cation signal intensity was observed in Figure 4.1 for a Tollen's thin silver film thickness that had a spectrophotometric

absorbance of approximately 0.6. Figure 4.5 demonstrates that the maximum $[\text{gramicidin S} + \text{Ag}]^-$ cation signal with respect to the Ag^- cation is largest for the smallest Tollen's thin silver film thickness used. Initially these conclusions seem contradictory, but they can be rationalized by considering the methods used to validate them. To reiterate, the laser fluence was included in the analysis of Figure 4.1, and the $[\text{gramicidin S} + \text{Ag}]^-$ cation signal was not compared to the internal standard Ag^- cation signal. Hence, the $[\text{gramicidin S} + \text{Ag}]^-$ cation signal was considered to be dependent on the laser fluence, and all the $[\text{gramicidin S} + \text{Ag}]^-$ cation signals were averaged at different laser fluences for one silver film thickness. However, Figure 4.5 did not consider the $[\text{gramicidin S} + \text{Ag}]^-$ cation signal to be dependent on the laser fluence, and the $[\text{gramicidin S} + \text{Ag}]^-$ cation signal was compared to the Ag^- cation. Hence, the laser fluence was not included in the analysis, even though the $[\text{gramicidin S} + \text{Ag}]^-$ and Ag^- cation signal intensities were taken at a variety of laser fluences. These assumptions are vital in understanding the analytical chemistry and the gas-phase ion chemistry that occurs during the LDI/LA process.

During LDI/LA, chemical ionization of the sample molecules occurs within the ablation plume to result in a $[\text{M} + \text{Ag}]^-$ peak. If Figure 4.5 is viewed as a sensitivity plot, then the $\Delta [\text{M} + \text{Ag}]^-$ -to- ΔAg^- ratio changes with the Tollen's thin silver film thickness. As the thickness of the Tollen's thin silver film decreases, this ratio increases. On the other hand, since a maximum $[\text{gramicidin S} + \text{Ag}]^-$ signal was observed at a Tollen's thin silver film thickness with an absorbance of 0.6, a specific $\Delta [\text{M} + \text{Ag}]^-$ -to- ΔAg^- ratio must exist for optimal gas-phase reactions within the ablation plume. This is analogous to MALDI where a specific matrix-to-analyte ratio must be attained for the best possible

TOFMS spectrum (Beeson et al., 1995).

Using the internal standard method, a linear relationship describes the dependence of a Δ [Sample + Metal]⁺ cation signal intensity (normalized to the Δ [Metal]⁺ cation signal intensity) and the thickness of the metal film (hence metal concentration within the ablation plume). Even though a given metal may not initially seem to react with a sample molecule, Figure 4.5 indicates that changes in the metal film thickness can affect the observation of the [Sample + Metal]⁺ cation in the LDI/LA-TOFMS. These results have shown that the [gramicidin S + Ag]⁺ cation formation efficiency can potentially be the largest using thin silver films. More importantly, however, the [gramicidin S + Ag]⁺/Ag⁺ ratio is more reproducible for thin silver films than thick silver films. Clearly, precise measurements of the silver cation affinity can be performed for silver films that are relatively thin.

4.2 Mechanism of LDI/LA-TOFMS

4.2.1 Experimental Details

The mechanism of our LDI/LA technique was next evaluated to provide both an insight into chemical species formation, and a reason for the upper mass detection limit. Back-illumination LDI/LA-TOFMS spectra of a thin silver film and gramicidin S deposited on a thin silver film were obtained using the third and fourth configurations (refer to Figures 2.9 A and 2.13 A). The parameters set on the LDI/LA-TOFMS instrument are listed in Table 4.3. Over 50 mass spectra were summed and averaged.

Table 4.3. Operational settings of the LDI/LA-TOFMS instrument using the third and fourth configurations.

Parameter	Value
Pressure P (torr)	1.5×10^{-6}
Laser wavelength (nm)	532
Laser fluence (mJ/cm^2)	14-250
Laser repetition rate (Hz)	1
Laser pulse width at FWHM (ns)	8
Lens focal length (cm)	25
Drift tube length D (cm)	42.2
Length s (cm)	variable
Length d (cm)	variable
Grid a voltage (kV)	20
Grid b voltage (kV)	0
Grid c voltage (kV)	0
Front-face voltage on MCP (kV)	-1.6

A 10- μ L aliquot of 4 mM gramicidin S solution in methanol was applied to each 2.5 cm x 2.5 cm Lumonics or Tollen's thin silver film. After air drying, the sample was placed inside the ion source chamber of the LDI/LA-TOFMS spectrometer for analysis.

4.2.2 Ion Generation

The LDI/LA mechanism can be simply put into two possible categories: a) direct desorption of sample/metal ions from the thin silver film, and b) chemical ionization. The configuration, and hence orientation of the thin silver film, can help elucidate the mechanism by producing different mass spectra. These observations are combined for discussion below.

Using the fourth configuration, the only mass spectral signal observed was assigned the Ag^+ cation. Hence, this cation was most likely directly desorbed from the thin silver film after it absorbed the incident laser energy. More interestingly, using the third configuration, $[\text{Ag}_n]^+$ cluster cations and pseudomolecular gramicidin S cations were produced. This suggests that gas-phase ion reactions occurred between the directly desorbed Ag^+ monomer cations and the neutral silver atoms/clusters or neutral gramicidin S molecules within the ablation plume:

When the complex is formed, the energy released is transferred to specie, X within the ablation plume via gas-phase ion-molecule reactions. Notice that multi-photon ionization of neutral gramicidin S molecules did not occur. Equation 4.2.2-2 likely applies to any sample deposited on the thin silver film surface. Since the Ag^+ monomer cation must travel through the ablation plume of neutral particles to allow for gas-phase ion reactions, the Ag^+ cation is not entirely produced at the thin silver film surface. This analysis is similar to that given by Cromwell et al. where laser ablated metal ions must travel

through a desorption cloud (Cromwell et al., 1993). Furthermore, the cross-section for the reaction between the metal ions and sample molecules is strongly dependent on the relative kinetic energy of the reactants (Halle et al., 1983).

When a potential was applied to the thin silver film in the third configuration, the Na^+ , Ca^+ and Ag^+ cations were present in the ablation plume. Neutral atoms/clusters also are present within the ablation plume (Haglund, 1996). As illustrated in Figure 4.6 A, the Ag^+ cations travel along the drift tube axis through the ablation plume where gas-phase ion-molecule reactions can occur. These reactions are responsible for the production of $[\text{Ag}_n]^+$ cluster cations and sample cation. However, using the fourth configuration, the generated Ag^+ cations must travel along the drift tube axis, perpendicularly away from the lateral/horizontal ablation plume, as shown in Figure 4.6 B. Hence no significant interaction with the neutrals can occur to form $[\text{M}+\text{Ag}]^+$ and $[\text{Ag}_n]^+$. The chemical ionization mechanism is further supported by the previous observation of $[\text{Ag}_n]^+$ ($1 \leq n \leq 20$) cluster cations by post-ionization experiments (Wucher, 1994). The $[\text{Ag}_n]$ clusters have ionization potentials around or slightly below 6.4 eV (Bonacic-Koutecky et al., 1999). The $[\text{Ag}_n]$ clusters should therefore be as easily ionized as a neutral Ag atom. However, the Ag^+ cation always had a relative abundance larger than those of $[\text{Ag}_n]^+$ cluster cations, probably due to the distribution of $[\text{Ag}_n]$ ($1 \leq n \leq 2$) neutral particles.

The 532-nm LDI/LA-TOFMS and 355-nm LDI/LA-TOFMS spectra of the Ag_2^+ cluster cation illustrated a different abundance. This observation is analogous to the internal excitation of silver clusters sputtered from a polycrystalline silver surface by Ar^+ ions for laser post-ionization of the ejected neutral species and TOFMS (Wucher, 1994). In that study, silver cluster cations (up to $n=20$) were generated with laser wavelengths of

Figure 4.6 LDI/LA production of Ag^+ monomer cations and neutral Ag_n atoms/clusters from the thin silver film using (A) the third configuration, and (B) the fourth configuration.

193 nm (6.4 eV) and 157 nm (7.9 eV). The 193-nm laser wavelength produced the Ag_6^+ cluster cation at an abundance lower than that for the 157-nm laser wavelength. This difference was attributed to a lower internal energy of the neutral Ag_6 clusters when using the 193-nm laser wavelength. Similarly, the difference in the Ag_2^+ cluster cation abundance of our LDI/LA technique can be explained because the neutral Ag atom has a higher internal energy when using the 355-nm laser wavelength. Consequently, the reaction described by Equation 4.2.2-1 occurs ($n=2$) and more Ag_2^+ cluster cations are produced. It is also important to realize that our LDI/LA spectra exhibited $[\text{Ag}_n]^+$ cluster cations for a maximum n of 9, which was significantly lower than that observed by Wucher. The higher internal energy of neutral Ag had a very significant impact on the silver cationization of bio-organic compounds because their 532-nm and 355-nm LDI/LA-TOFMS spectra were usually different. This observation provides further insight into the LDI/LA process. The larger internal energy of the neutral Ag atom acquired when using a 355-nm laser wavelength was likely transferred to the bio-organic molecules via gas-phase reactions:

Since chemical ionization occurred, the neutral bio-organic molecules that had a higher internal energy (M^*) further reacted with the Ag^+ cation via Equation 4.2.2-2. In this sense, the larger internal energy of the bio-organic molecules can play an important role in their efficient silver cationization.

4.2.3 Laser Energy Dependence

Other research groups had previously suggested that the probability of $[\text{Ag}_n]^+$ cluster cation generation during FAB experiments increased with increasing temperature (Summer et al., 1989; Szekely and Allison, 1997). However, the generation of cluster cations in the present LDI/LA experiments did not appear to correlate with the laser fluence (and hence temperature). The $[\text{Ag}_n]^+$ cluster cation peaks that appeared at low laser fluences were most likely formed by the softer LDI/LA process of electronic excitation. Gotz et al. first proposed that non-thermal LDI/LA of sodium atoms induced by the absorption of 355-nm and 532-nm wavelengths resulted in an electronic excitation process, while adsorption of a 1064-nm wavelength resulted in a thermal process (Gotz et al., 1996 B). The electronic excitation caused transition to an anti-bonding orbital. At high laser fluences in the present LDI/LA experiments, the $[\text{Ag}_n]^+$ cluster cations were thermally desorbed. According to a 1-dimensional model, the thin silver film's backside temperature eventually increased to 900 ± 200 K (Nishigaki et al., 1979), which came close to silver's melting point of 1234 K. Similar LDI/LA of a thin gold film using a 337-nm wavelength resulted in the generation of $[\text{Au}_n]^+$ ($1 \leq n \leq 3$) cluster cations (Huang et al., 1988), which was eventually attributed to the thermal desorption mechanism (Standing and Ens, 1991). Clearly, the choice of metal (sodium versus gold) influenced the mechanism. Since the present Tollen's thin silver films were non-uniform, electronic-excitation desorption is expected (Dickinson et al., 1994; Hoheisel et al., 1993; Hattori et al., 1991; Vollmer and Trager, 1987; Nishi et al., 1984), and it is not unreasonable to observe $[\text{Ag}_n]^+$ cluster cation peaks of random intensities.

Although the electronic excitation process during our present LDI/LA mechanism was not fully investigated, the non-thermal desorption process of molecules from metal surfaces had previously been investigated by other researchers. Direct evidence had been reported for the existence of at least two different desorption modes, non-thermal and thermal, depending on the laser power density and the sample thickness (Linder and Seydel, 1985). There was also evidence supporting a non-thermal ablation of nickel and gold films with 8-ps pulses (Rosenfeld and Campbell, 1996). For the laser ablation of sodium, non-thermal pathways are evident under low laser fluence, but thermal pathways dominate under high laser fluence (Gotz et al., 1996 B). Even photo-stimulated desorption of ammonia from copper using the 193-nm and 308-nm wavelengths confirmed electronic excitation by the linear laser power dependence of the desorption yield (Hertel et al., 1995).

The contribution of thermal desorption to our LDI/LA process was evaluated by monitoring the change in TOFMS peak width with temperature. It had been shown that laser ablated metal ions were generally in high-temperature states with broad internal and kinetic energy distributions (Kang and Beauchamp, 1985). In a recent report, time-of-flight distributions of laser desorbed PEG oligomers were fitted to a Maxwell-Boltzmann distribution (Handschuh et al., 1997). With increasing laser fluence and hence temperature, the distributions became broader. Clearly, the width (and hence resolution) of our gramicidin S peak could be monitored as a function of the laser fluence to determine if our LDI/LA process was indeed thermal in nature. As shown in Figure 4.7, the pseudomolecular gramicidin S cation signal evolves from a narrow peak to a broad one as the laser fluence is increased. The gramicidin S resolution ($M/\Delta M$) decreases

Figure 4.7 Changes in the pseudomolecular gramicidin S cation signal width when the laser fluence increases from 85 mJ/cm² to 396 mJ/cm².

Figure 4.8 Plot of Gramicidin S resolution against laser fluence (mJ/cm²).

non-linearly with laser fluence as shown in Figure 4.8, but it appears linear over a rather small range of fluences. These results confirm the thermal nature of our LDI/LA process at high laser fluences.

As thermal conductance varied with distance from the thin silver film backside to the film surface, a delicate balance between the electronic excitation and thermal desorption was expected. Since the melting and ablation thresholds of thin films can be predicted by the thermal diffusion length and optical penetration depth (Matthias et al., 1994), both of these LDI/LA processes (electronic excitation and thermal desorption) must also depend on the thermal diffusion length and optical penetration depth. Also, the laser pulse length was crucial for the ablation efficiency and hence the degree of thermal damage adjacent to the ablated area (Siegel et al., 1997). Furthermore, film melting at high laser fluences during LDI/LA might affect a variable fraction of the total thickness of the thin film (Toth et al., 1995). Lastly, LA thresholds could vary significantly with

two or more different metal film layers as evidenced by a gold-chromium double layer on fused silica that behaved optically like gold and thermally like chromium (Matthias et al., 1994).

It has recently been reported that the thermal diffusivity of silver, which is needed to calculate the thermal diffusion length, can increase with both temperature and grain size of silver (Du and Wu, 1996). With increasing laser fluence, the silver film surface temperature increases, and consequently the thermal diffusivity and the thermal diffusion length will increase. However, at constant laser fluence, if the thickness of the thin silver film increases, the proportion of the thermal diffusion length to the silver film thickness decreases, and the silver film surface temperature most likely decreases. Since the cited reference only considers thin silver films (200 – 800-nm), one can expect that the model will break down when the silver film becomes too thick (the silver must be treated as a bulk material).

Since the optical penetration depth of metal films is typically 1/20 of the chosen wavelength (Matthias et al., 1995), the optical penetration depth for our thin silver films was approximately 26 nm when a 532-nm wavelength was used for LDI/LA. Thus, LDI/LA of a silver film whose thickness was less than this optical penetration depth was most likely a consequence of an electronic-excitation process. As the thickness of the thin silver film increases, the proportion of the optical penetration depth to the silver film thickness decreases, and the effect of the wavelength on the LDI/LA process decreases in regards to electronic excitation. At a low laser fluence (low silver film surface temperature) with a silver film thickness smaller than the optical penetration depth, LDI/LA most likely occurs via electronic excitation. At this fluence, the thermal

diffusion length is small and thermal desorption does not occur. However, upon using high laser fluences at the same silver film thickness, the thermal diffusion length increases and thermal desorption starts to dominate. For a silver film thickness that is greater than the optical penetration depth, LDI/LA most likely occurs via thermal desorption only. In fact, LA of silver films greater than 60- μm thick exhibited partial melting, complete melting, boiling and even complete vaporization (Andrew et al., 1983).

Chapter 5 SPR-LDI/LA-TOFMS of Gramicidin S

5.1.1 Experimental Details

The effect of SPR on our LDI/LA technique was evaluated. The absorption of p-polarized 532-nm wavelength was expected to lower the laser fluence required to generate a mass spectrum. SPR-LDI/LA-TOFMS spectra of a thin silver film and gramicidin S deposited on a thin silver film were analyzed using the fifth configuration (refer to Figure 2.14 A). The parameters set on the LDI/LA-TOFMS instrument are listed in Table 5.1. 50 mass spectra were summed and averaged mass spectra to improve the signal-to-noise ratio. A peak was recognized when its signal-to-noise ratio was greater than 10:1.

Table 5.1. Operational of the SPR-LDI/LA-TOFMS instrument.

Parameter	Value
Pressure P (torr)	5×10^{-7}
Laser wavelength (nm)	532
Laser fluence (mJ/cm^2)	30-70
Laser repetition rate (Hz)	1
Laser pulse width at FWHM (ns)	8
Drift tube length D (cm)	42.2
Length s (cm)	2.0
Length d (cm)	-
Grid a voltage (kV)	20
Grid b voltage (kV)	0
Grid c voltage (kV)	-
Front-face voltage on MCP (kV)	-1.6

A 10- μ L aliquot of 4.0 mM gramicidin S solution in methanol was applied to a 2.5 cm x 2.5 cm Lumonics thin silver film. After air drying, a SPR angular profile was taken to ensure that SPR activity existed. The dry sample was placed inside the ion source chamber of a TOFMS spectrometer for SPR-LDI/LA analysis.

A 250- μ L aliquot of 0.2 mM gramicidin S solution in methanol and a 250- μ L aliquot of 65 mM 2,5-dihydroxybenzoic acid (DHB) solution in ethanol were deposited on a 2.5 cm x 2.5 cm Lumonics thin silver film. After air drying, a SPR angular profile was taken to ensure that SPR activity existed. The dry sample was placed inside the ion source chamber of a TOFMS spectrometer for SPR-LDI/LA analysis.

5.1.2 Characterization

The thin silver film's SPR activity was tested external to the SPR-LDI/LA-TOFMS system using a red light emitting diode source, a polarizer, a glass prism in the Kretschman geometry(Kretschmann, 1971), and a photodiode array detector. A dip in the SPR angular profile would indicate SPR activity. The SPR angular profiles for a thin silver film, a gramicidin S sample deposited on a thin silver film, and a gramicidin S/DHB matrix mixture deposited on a thin silver film are shown in Figure 5.1. Upon deposition of gramicidin S onto the thin silver film surface, a significant shift in the SPR angle from that for a thin silver film occurred and SPR activity lessened as indicated by the higher reflectance. The SPR angle for gramicidin S with a DHB matrix was only slightly shifted, but the dip became less pronounced. The overall reflectance was significantly lower than the reflectance for either a thin silver film or gramicidin S deposited on a thin silver film. These key features described for the SPR angular profiles for each sample were reproducible. They were the result of the sample thickness on the thin silver film surface, the optical properties of the sample (as compared to air), and the sample morphology (an non-uniform layered film).

Figure 5.1 SPR angular profiles for a thin silver film, a gramicidin S sample deposited on a thin silver film, and a gramicidin S/DHB matrix mixture deposited on a thin silver film.

5.1.3 Laser Desorption Ionization/Laser Ablation Processes

The three processes that occurred during the SPR-LDI/LA mechanism were deformation, desorption, and ionization. Since the reflectance of the laser beam could not be measured directly inside the ion source chamber, these processes were observed upon visual inspection. Deformation on the backside of the thin silver film would yield abnormalities in the silver film, thought to occur from the large temperature increase, causing melting on the backside of the thin silver film. The desorption process would produce an ablation hole in the thin silver film. A measurement of desorption was the change in He-Ne laser light absorption by the thin silver film within each spot. A plot of the absorbance against the angle of incidence for the p-polarized 532-nm laser beam would indicate that maximum silver desorption occurred at the SPR angle. Lastly, the ionization process would generate a mass spectrum. The mass spectra of a thin silver film and a gramicidin S sample deposited on a thin silver film were recorded at various angles of incidence at a constant laser fluence. A graph of the Ag^+ cation signal intensity or pseudomolecular gramicidin S cation signal intensity normalized to the desorption hole size against the angle of incidence could illustrate enhanced ionization at the SPR angle.

5.1.4 Thin Silver Film

SPR-LDI/LA-TOFMS for a thin silver film produced identical mass spectral peaks as the LDI/LA-TOFMS results for a thin silver film, as described in Section 3.1. The abundance ratios of all relevant peaks might slightly vary, although the Ag^+ cation dominated the mass spectrum. The resolution ($M/\Delta M$) was approximately 15 for the Ag^+ cation peak, while its signal-to-noise ratio was 208:1. A minimum laser fluence of $33 (\pm 3) \text{ mJ/cm}^2$ was required for obtaining a Ag^+ cation signal at the SPR angle of $47.5 (\pm 1.5)^\circ$. Spectra obtained under off-resonance conditions required larger laser fluences. At a constant laser fluence of $55 (\pm 4) \text{ mJ/cm}^2$, the largest signal intensity for the Ag^+ cation was observed at the SPR angle, accompanied by the largest desorption/ablation hole size (as represented by the spectrophotometric absorbance), as seen in Figure 5.2. These results were in good agreement with the independent PAS signal amplitude measurements (not shown). The validity of SPR enhancing LDI/LA of a thin silver film was confirmed by a comparing the ablation hole size between s-polarized and p-polarized 532-nm wavelength laser light. The ablation hole size was represented as the mass of paper required to fill an enlarged projection of the thin silver film ablation hole. At the constant laser fluence of $55 (\pm 4) \text{ mJ/cm}^2$, no ablation hole due to silver desorption could be readily produced at any angle of incidence in the range of 38° to 51° using s-polarized light as shown in Figure 5.3. However, a maximum ablation hole was generated at $47.5 (\pm 1.5)^\circ$ using p-polarized light.

Figure 5.2 A plot of the Ag⁺ cation signal intensity and the laser desorption/ablation hole size (as represented by spectrophotometric absorption units) against the angle of incidence.

Figure 5.3 Variations of the ablation hole size, measured through the mass of the ablated hole, for a thin silver film using s-polarized and p-polarized 532-nm wavelength.

5.1.5 Gramicidin S

Using a thin silver film deposited with gramicidin S, SPR-LDI/LA-TOFMS indicated similar mass spectral peaks as those described in Section 3.2.3 for a thin silver film deposited with gramicidin S. The most abundant peaks were the Na^+ , Ca^+ and $[\text{Ag}_n]^+$ ($n \geq 1$) cluster cation signals, while a lower abundance was exhibited by the $[\text{gramicidin S} + \text{Na}]^+$ and the $[\text{gramicidin S} + \text{Ag}]^+$ signals. Fragmentation ion signals at m/z values ranging from 88 to 200 were also observed. The $M/\Delta M$ was 12 while the signal-to-noise ratio was respectively 10:1 and 5:1 for the pseudomolecular gramicidin S ions. In general, a greater laser fluence was required for any deformation of a gramicidin S sample deposited on a thin silver film than a thin silver film alone, the minimum laser fluence required ranging from 55 mJ/cm^2 to 73 mJ/cm^2 . This was expected because absorption of p-polarized 532-nm laser light by the thin silver film with a sample overlayer under SPR conditions was decreased, in accordance with Figure 5.1. This suggested that more energy would be required for the desorption/ablation. A minimum laser fluence of approximately 55 (± 4) mJ/cm^2 was required for LDI/LA at the SPR angle of 49.5 (± 1.5)°, which was lower than those required under off-resonance conditions. This laser fluence was significantly higher than the minimum laser fluence required for LDI/LA of a thin silver film at its SPR angle of 47.5 (± 1.5)°. Also, at the same laser fluence, a larger abundance of pseudomolecular gramicidin S signal was seen at the SPR angle of 49.5 (± 1.5)°. The largest pseudomolecular gramicidin S signal intensity also coincided with the largest desorption/ablation hole size, as shown in Figure 5.4.

Figure 5.4 A plot of the [gramicidin S+Ag]⁺ cation signal intensity and the laser desorption/ablation hole size (as represented by spectrophotometric absorption units) against angle of incidence.

5.1.6 Gramicidin S with a Dihydroxybenzoic Acid (DHB) Matrix

Gramicidin S mixed with a commonly used MALDI matrix, DHB, was deposited on a thin silver film for analysis. As shown in Figure 5.5, its SPR-LDI/LA-TOFMS spectrum exhibits all substrate ion signals and $[\text{gramicidin S} + \text{Na}]^+$, but not $[\text{gramicidin S} + \text{Ag}]^+$. Hence, it appears as if the matrix hindered the production of $[\text{gramicidin S} + \text{Ag}]^+$. The $M/\Delta M$ is approximately 12 while the signal-to-noise ratio is approximately 10:1 for the $[\text{gramicidin S} + \text{Na}]^+$ cation. Fragmentation ions from the gramicidin S and DHB are also observed in this mass spectrum. More interestingly, the minimum laser fluence required to generate the $[\text{gramicidin S} + \text{Na}]^+$ cation signal at its SPR angle of $48.0 (\pm 1.5)^\circ$ was $30 (\pm 10) \text{ mJ/cm}^2$, which is roughly the same as that required for a thin silver film. The minimum laser fluence requirement for observing a SPR-LDI/LA-TOFMS spectrum at the SPR angle appeared to mimic the low reflectance of the SPR angular profile shown in Figure 5.1. Apparently the addition of the DHB matrix isolated the gramicidin S from the thin silver film surface, reduced their adsorption interaction and thereby lowered the minimum laser fluence requirement during SPR-LDI/LA. Upon visual inspection of the thin silver film, the addition of a matrix helped desorb all the gramicidin S from the silver film surface, leaving no gramicidin S residue on the silver film surface after SPR-LDI/LA.

Figure 5.5 SPR-LDI/LA-TOFMS spectrum of gramicidin S with DHB matrix.

5.1.7 Ionization Efficiency

Table 5.2 is a summary of all the SPR-LDI/LA-TOFMS experimental results presented above. It lists the minimum laser fluence required for the successful desorption/ablation of a thin silver film, a gramicidin S sample deposited on a thin silver film, and a gramicidin S/DHB matrix mixture deposited on a thin silver film. This list includes LDI/LA-TOFMS back illumination experiments and LDI/LA-TOFMS front illumination experiments for comparison.

Clearly, the minimum laser fluence requirement varied among these (SPR)-LDI/LA-TOFMS techniques. However, SPR-LDI/LA always required a smaller laser fluence compared with the other techniques. Although SPR appeared to assist the

Table 5.2 Minimum laser fluence required to generate a mass spectrum under front-illumination LDI/LA conditions, back-illumination LDI/LA conditions, and back-illumination SPR-LDI/LA conditions for a thin silver film, and gramicidin S deposited with and without DHB matrix.

Sample	Minimum laser fluence requirement		
	LDI/LA-TOFMS front illumination (mJ/cm ²)	LDI/LA-TOFMS back illumination (mJ/cm ²)	SPR-LDI/LA- TOFMS back illumination (mJ/cm ²)
Thin silver film	50 ± 2	67 ± 1	33 ± 3
Gramicidin S on thin silver film	100 ± 5	76 ± 2	55 ± 4
Gramicidin S/DHB matrix mixture on thin silver film	>50	> 50	30 ± 10

LDI/LA process for gramicidin S, a final comparison between the ratio of signal intensity to ablation hole size and the angle of incidence must be performed. This comparison would clearly demonstrate whether ionization was enhanced during SPR.

The plot of the ratio of signal intensity to ablation hole size against the angle of incidence is illustrated in Figure 5.6. A maximum ratio value of silver signal to ablation hole size is observed at the SPR angle of $45.5 (\pm 1.5)^\circ$. This value lies within the experimental errors of SPR angles obtained separately for the maximum Ag^+ cation signal and the maximum ablation hole size. The maximum ratio demonstrates that SPR assisted in the ionization process. Similarly there is a maximum ratio of gramicidin S signal to ablation hole size at the corresponding SPR angle of $49.0 (\pm 1.5)^\circ$.

Figure 5.6 Plot of the ratio of signal intensity to desorption/ablation hole size (as represented by spectrophotometric absorption units) against the angle of incidence.

5.1.8 Spatial Effects during the SPR-LDI/LA Mechanism

The resolution of a TOFMS spectrum can reveal the LDI/LA energetics, including those for the present SPR mechanism. Investigations on angular distributions of silver ions and neutrals by laser ablation had previously shown that most Ag^+ cations were found along the normal (Hansen et al., 1997). Since all the LDI/LA-TOFMS spectra had comparable resolutions, a general LDI/LA mechanism may be applied to all film/analyte combinations. However, using the fourth configuration the LDI/LA-TOFMS spectra of thin silver film with and without a deposited sample had a resolution that was higher than the SPR-LDI/LA-TOFMS spectra, as illustrated in Figure 5.7. Clearly, the energy dissipated from a thin silver film backside to the film surface was different under SPR conditions. It had previously been suggested that silver clusters on a glass plate absorbed laser light to excite surface plasmon modes of electrons in the clusters (Kim et al., 1994 B). The electronically excited silver surface plasmon mode then lost energy by desorbing the silver surface clusters and any absorbed analyte molecules (Lee et al., 1993). In the present SPR-LDI/LA work, the thin silver film absorbed the laser energy via SPR excitation, enhanced the LDI/LA, and thus lowered the laser fluence requirement. It had also been reported that p-polarized radiation with a 532-nm laser beam stimulated desorption more efficiently by 30% than s-polarized light because the p-polarized laser radiation predominantly interacted with a dipole in the direction normal to the surface of the oblate clusters (Gotz et al., 1996 A).

Since the spectra obtained in the fourth configuration indicated that the resolutions during the SPR-LDI/LA process were less than the resolutions during the

Figure 5.7 (A) A typical LDILA-TOFMS spectrum, and (B) a typical SPR-LDI/LA-TOFMS spectrum of a thin silver film.

LDI/LA process, the absorption of p-polarized laser light by the thin silver film during SPR appeared to increase the spatial and/or kinetic energy distributions of the Ag^+ monomer cations and neutrals. A depiction of the SPR-LDI/LA process is illustrated in Figure 5.8. Figure 5.8 A shows that SPR increased the angular distribution of desorbed silver ions and neutrals. When ablating the thin silver film in the fifth configuration, the spatial and kinetic energy distributions along the drift tube axis were relatively small, as shown in Figure 5.8 B.

Figure 5.8 SPR-LDI/LA production of Ag^+ monomer cations and neutral Ag_n atoms/clusters from the thin silver film using: (A) the fourth configuration, and (B) the fifth configuration.

Chapter 6 Conclusions

A Wiley-McLaren TOFMS model, written in EXCEL, was employed to determine the distances s (0.26 cm) and d (2.60 cm) for optimum resolution, given a drift tube length D of 42.2 cm. This allowed the design and construction of five configurations for a LDI/LA-TOFMS instrument. Unfortunately, arcing occurred frequently and the determined theoretical distances could not be utilized. However, the overall resolution of the instrument improved upon using higher grid voltages. Additional modifications were performed based on the successful detection of the model compound gramicidin S. The third configuration was primarily suited for LDI/LA-TOFMS experiments because the upper m/z limit was improved to 6000 Da (a factor of five), lighting problems were eliminated, and the incorporation of a high voltage feedthrough provided better MCP detection.

The LDI/LA technique was used for molecular mass and structural reactivity analysis. A sample was deposited by solvent evaporation on the thin silver film and was subjected to a 355-nm or 532-nm laser beam from a Nd-Hyper YAG laser by back-illumination. Silver cluster cations $[Ag_n]^+$ ($1 \leq n \leq 3$) were primarily observed in the mass spectrum and used to calibrate the time scale to construct a LDI/LA-TOFMS spectrum. This technique has been successfully applied to dithizone, 1,4,8,11-tetraazocyclotetradecane, dicyclohexyl-18-crown-6 ether, [5]-helicene dendrimer, gramicidin S, substance P, and mellitin. Further experiments confirmed that these bio-organic molecules reacted in the gas phase to produce M^+ , $[M+Ag]^+$ and/or $[M+Na]^+$ cations. A variety of PAH compounds, fullerenes and fullerene derivatives were tested

with the present LDI/LA technique. Their results illustrated electron-transfer reactions occurring via gas-phase ion-molecule reactions. Furthermore, charge-remote fragmentation of a variety of thia fatty esters and acids, tested with the present LDI/LA technique, was thought to occur. One advantage of using a thin silver film over conventional LDI/LA techniques is that the sample does not need to have appreciable spectral absorption at the laser wavelength.

The physical process that occurs during the present LDI/LA technique was elucidated with internal standardization and ion association using gramicidin S. For a given silver film thickness, the intensity of the $[M+Ag]^+$ cation signal varied, but the $\Delta[M+Ag]^+/\Delta Ag^+$ ratio did not. When the silver film thickness was increased, this ratio decreased. The maximum $[M+Ag]^+$ cation signal was obtained for a silver film thickness with a spectrophotometric absorbance of 0.6, that corresponds to a thickness of approximately 20 nm. The LDI/LA mechanism that generated the $[M+Ag]^+$ cation was electronic excitation at low laser fluences and thermal excitation at high laser fluences.

The coupling between SPR and LDI/LA for TOFMS was investigated using the Krestchman geometry. At the SPR angle θ_r , the thin silver film absorbed the energy of incident laser photons. Based on a Basic computer program, the SPR angle, θ_r was determined to be 43° for a 532-nm wavelength. A Fortran computer program, written to better model the SPR phenomenon, tabulated strong absorption of the 532-nm wavelength at a θ_r of 44° . Furthermore, an optimum silver film thickness was determined to be 40-60 nm, which was based on the lowest reflectance of the 532-nm laser beam by the thin silver film at the θ_r . Reflectance and photoacoustic measurements

confirmed the SPR modeling results and were further employed to establish θ_r during SPR-LDI/LA-TOFMS experiments.

The fifth configuration was only suitable for SPR-LDI/LA-TOFMS experiments because it provided precise and accurate laser energy and fluence readings. The SPR-LDI/LA technique demonstrated that a lower minimum laser fluence for the production of the silver cluster cations $[\text{Ag}_n]^+$ was required at the θ_r . This is in accordance with the silver surface plasmon of energy 3.7 eV assisting the LDI/LA process. Even when gramicidin S was deposited on the surface of the thin silver film, there was a lower minimum laser fluence at its θ_r . The $[\text{M}+\text{Na}]^+$ and $[\text{M}+\text{Ag}]^+$ cation signals were the result of gas-phase ion-molecule reactions. When a 2,5-dihydroxybenzoic acid was applied prior to deposition of the gramicidin S, an even lower laser fluence was required, which was attributed to matrix isolation. SPR assisted in the electronic excitation desorption during LDI/LA of a thin silver film. The capability to perform SPR-LDI/LA on a 1141 Da molecular mass represents a new milestone beyond previous achievements. Future work will investigate the proper selection of matrices for even larger molecular masses in SPR-LDI/LA-TOFMS experiments.

Appendix 1: Operation of the Lumonics Nd-Hyper YAG Laser

To start the laser, the following procedure is typical:

1. Connect the laser power supply to the mains. Ensure that the circuit breaker and combined earth leakage detector of the power supply tower is ON (up). Rotate the red mains isolator switch to position 1 (on). The oscillator OFF and shutter CLOSE LED's will light up on the LCU.
2. Flip the cooler switch (at the back of the power supply tower) to ON. The switch will light up (green) and the cooler pump will start.
3. Allow 30 min for the Pockels cell and HGA to reach operating temperature.
4. For operation in the Q-switched mode, ensure the Pockels cell ON button is depressed and the REP RATE is selected.
5. Turn the power keyswitch on the LCU clockwise to the ON position. A further turn to the biased START position illuminates the emission indicator on the LCU. If the emission indicator does not remain lit when the key switch is released from its START position, an interlock fault is indicated.
6. After a 5-s delay, the power supply is enabled and the oscillator ON LED will light up, indicating that the simmer arc has been established in the flashlamp.
7. Press the oscillator ON button; the flashlamp inside the Nd-Hyper YAG laser will start pulsing.
8. Check along the imaginary beam path to ensure there are no unwanted obstacles and that the beam will be safely contained. Remove the knurled safety end cap from the appropriate laser output port.

9. Laser output is now available by pressing the shutter OPEN button to operate the intercavity shutter.

To terminate the laser operation, the following procedure is typical:

1. Press the shutter CLOSE button and the oscillator OFF button. The flashlamp will stop pulsing, and there will be no beam coming out from the laser output port.
2. Close the safety end cap(s).
3. Turn the keyswitch on the LCU to the OFF position ,and remove the key if necessary.
4. Wait for at least 20 min, then flip off the cooler switch (at the back of the power supply tower) and turn off the cooling water supply.

Appendix 2: Operation of the TOFMS

2.1 Operation of the High Vacuum System

The procedure for pumping down the high vacuum system is as follows:

1. Turn on the water flow to the diffusion pump coils (at the sink).
2. Flip the red switch up to turn on the vacuum safety unit.
3. Close all system valves.
4. Turn on the Daia Sinku rotary pump.
5. Open the (lower) backing valve. This rough pumps the diffusion pump chamber down to a pressure of 10^{-3} torr.
6. Switch on the diffusion pump heater.
7. Switch on the ionization gauge controller and allow 30 minutes to warm up the electronics.
8. Close the backing valve and slowly open the (upper) roughing valve. This rough pumps the vacuum chamber to a pressure of 10^{-3} torr.
9. Close roughing valve and re-open the backing valve to ensure the pressure between the diffusion pump chamber and the vacuum chamber is the same. Slowly open the gate valve.
10. Engage the thermal interlock safety switch on the ionization gauge controller (Varian 971-0003). A base pressure of 10^{-7} torr in magnitude should be displayed on the analogue meter.

The procedure for terminating pumping is as follows:

1. Press the ionization gauge filament OFF button and turn the main power switch to OFF.
2. Close the gate valve.
3. Switch off the diffusion pump heater.
4. 5. After 3-4 hours, close the backing valve to isolate the diffusion pump. This allows the diffusion pump to cool.
6. Vent the vacuum chamber.
7. Turn off the water to the diffusion pump coils.
8. Switch off the vacuum safety unit.

2.2 Operation of the (SPR)-LDI/LA-TOFMS

The procedure for turning on the (SPR)-LDI/LA-TOFMS is as follows:

1. Turn on the Nd-Hyper YAG laser (see Appendix 1 on page 223).
2. Insert a thin silver film to the sample holder
3. Make all the necessary internal electrical connections.
4. Evacuate the (SPR)-LDI/LA-TOFMS system (see Appendix 2.1 on page 225).
5. Once a base pressure of approximately 10^{-6} to 10^{-7} torr is achieved, inspect carefully to ensure all electrical connections are not shorted.
6. Place all the necessary optical equipment (prisms, focusing lens, etc.) at their proper positions.
7. Ensure the external electrical connections to power supplies, fast photodiode, DSO, etc. are appropriately connected and not shorted.
8. Turn on power supplies for the fast photodiode, DSO, etc.
9. A mass spectrum will be recorded once the shutter OPEN button is pressed. These spectra can be saved on a 3.5" disk if the LeCroy 9310 M DSO is used.

The procedure for turning off the (SPR)-LDI/LA-TOFMS is as follows:

1. Turn off the Nd-Hyper YAG laser (see Appendix 1 on page 224).
2. Turn off all power supplies for the DSO, fast photodiode, etc.
3. Close the gate valve and open the vent.
4. Once at room pressure, remove the top flange, sample and internal electrical connections with caution.
5. To turn the (SPR)-LDI/LA-TOFMS on again, follow steps 2, 8, 9, and 10 from Appendix 2.1 on page 225.
6. Follow the remaining steps from Appendix 2.2 on page 227, starting from step 5.

Appendix 3: Programs

3.1 Basic Program for determining relationship between SPR angle and wavelength

```

10 REM CALCULATION OF ANGLE OF INCIDENCE FOR VARYING LAMBDA
20 REM MAY INPUT VALUES FOR DIELECTRICS OF SAMPLES
21 REM DEFINE VARIABLES
22 REM E1 IS REAL DIELECTRIC OF METAL (FUNCT OF FREQUENCY)
23 REM E2 IS DIELECTRIC OF SAMPLE
25 PRINT "RELATIONSHIP BETWEEN INCIDENCE ANG AND WAVELENGTH"
26 INPUT E2,E3
27 PRINT "SAMPLE HAS DIELECTRIC OF "; E2
29 PRINT "DIELECTRIC OF PRISM IS "; E3
30 PRINT
35 PRINT "WAVELENGTH", "ANGLE OF INCIDENCE "
40 PRINT "(NANOMETERS)", " (DEGREES)"
45 PRINT
46 RESTORE
55 FOR M = 1 TO 21 STEP 1
60 READ E,N,K
65 L = ((6.626E-34)*(3E+17))/((1.6022E-19)*(E))
70   E1 = (N*N) - (K*K)

```

```
75    B = SQR ((E1*E2)/(E3*(E1+E2)))
80    C = ATN (B/(SQR(1-(B*B))))
85    A = (57.3)*C
90    PRINT L,A
95    NEXT M
100 PRINT " DO YOU WISH TO INPUT NEW VALUES ?"
105 INPUT XS
110 IF XS = "YES" THEN 26
125 DATA 1.39,0.04,6.312, 1.51,0.04,5.727, 1.64,0.03,5.242
130 DATA 1.76,0.04,4.838, 1.88,0.05,4.483, 2.01,0.06,4.152
135 DATA 2.13,0.05,3.858, 2.26,0.06,3.586, 2.38,0.05,3.324
140 DATA 2.50,0.05,3.093, 2.63,0.05,2.869, 2.75,0.04,2.657
145 DATA 2.88,0.04,2.462, 3.00,0.05,2.275, 3.12,0.05,2.070
150 DATA 3.25,0.05,1.864, 3.37,0.07,1.657, 3.50,0.10,1.419
155 DATA 3.62,0.14,1.142, 3.74,0.17,0.829, 3.87,0.81,0.392
160 DATA 3.99,1.13,0.616, 4.12,1.34,0.964, 4.24,1.39,1.164
165 DATA 4.36,1.41,1.264, 4.49,1.41,1.331, 4.61,1.38,1.372
170 END
```


3.2 Fortran Program for determining relationship between reflectance and angle of incidence

Defining the variables

Complex kx0,kz0,kz1,kz2,kz3
 Complex r01,r12,X0,X1,X2,e0,e1,e2,ti
 Complex r02num,r02den,r02
 Real d1,ref(20),ds,de,di,dc
 integer i, j, k, m, nodata
 real fps,as,ae,ai,choice,cv,ep
 real er(20), ei(20), l(20), angle(180)
 character filename*12

Enter the file name that has the dielectric constants of medium 0 and 2, and the thickness of the thin silver film in nm.

print*,'Enter file for data'
 read*,filename
 open(unit=3,file=filename)
 read(3,*)e0
 read(3,*)e2
 read(3,*)d1

```
close(unit=3)
```

In a pre-existing file "ag.txt", there are three numbers that corresponds to the real and imaginary dielectric constant of silver for a given wavelength. Each set of three corresponds to one "number of data". Enter the number of data that the user desires. This will tell the program to read "number of data" set.

```
print*, 'Enter the number of data '
read*, noda
open(unit=3, file='ag.txt')
do i=1, noda
  read(3, *) er(i), ei(i), l(i)
  l(i)=l(i)*1E-6
enddo
close(unit=3)
```

The reflectance and angle of incidence data are stored in a file that the user enters below.

```
print*, 'Data saved as which file '
read*, filename
open(unit=3, file=filename)
```

Setting the initial and final angle of incidence, and prompting the user for the angle of incidence increment.

```

as=0

ae=90

print*,'Enter angle increment '

read*,ai

```

Calculating the k_z and k_x wave vectors given the above information. Then, the reflectance is calculated. The angle of incidence and the reflectance are exported as columns to the file that the user requested.

```

i=1

angle(i)=as

while(angle(i).le.ae)do

    angle(i)=angle(i)*3.14/180

    print*,'angle ',angle(i)*180/3.14

    do j=1,nodata

        e1=cplx(er(j)**2-ei(j)**2,2*er(j)*ei(j))

        fps=4*(3.14**2)

        kz0=csqrt((e0*fps)/(l(j)**2)/

* (1+(tan(angle(i)))**2))

        kx0=kz0*(tan(angle(i)))

        kz1=csqrt((e1*fps)/(l(j)**2)-(kx0**2))

        kz2=csqrt((e2*fps)/(l(j)**2)-(kx0**2))

```

```

X0=kz0/e0
X1=kz1/e1
X2=kz2/e2
r01=(X0-X1)/(X0+X1)
r12=(X1-X2)/(X1+X2)
ti=cmplx(0,2)
r02num=r01+r12*cexp(ti*kz1*d1)
r02den=1+r01*r12*cexp(ti*kz1*d1)
r02=r02num/r02den
ref(j)=cabs(r02)
write(3,*) l(j),angle(i)*180/3.14,ref(j)
enddo
angle(i)=angle(i)*180/3.14 +ai
endwhile
close(unit=3)

```

3.3 Fortran Program for converting an oscilloscope file to an EXCEL file

To run this program, the files lecroy21.tpl, wavetran.exe and oscilloscope files must be in the same directory as this file.

```
integer i, initwave, finalwave
```

Enter the initial and final file numbers (the oscilloscope stores files as * ###)

```
print*, 'Initial wave file number : '
```

```
read*, initwave
```

```
print*, 'Final wave file number : '
```

```
read*, finalwave
```

```
open(unit=3, file='dowatr.bat')
```

```
do i=initwave, finalwave
```

```
  if (i.lt.10) then
```

```
    write(3,5)i,I
```

Entering a line in the file "dowatr.bat" in sequential order

```
5  format (1X, 'wavetran -tlecroy21.tpl -oscl dat.00', I1, ' sc1.00',
```

```
  * i1)
```

```
    elseif (i.lt.100.and.i.gt.9) then
```

```
      write(3,6)i,i
```

```
6  format (1X, 'wavetran -tlecroy21.tpl -oscl dat.0', i2, ' sc1.0',
```

```
  * i2)
```

```
    else  
        write(3,7)i,i  
7    format (1X, 'wavetran -tlecroy21.tpl -osc1 dat.',i3,' scl.',  
    * i3)  
        endif  
    enddo  
    close(unit=3)  
end
```

Run the file "dowatr.bat" and the files will be readable by EXCEL

References

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