

Laser-induced acoustic desorption

Xin Ma, Yuyang Zhang, Hao-Ran Lei, and Hilkka I. Kenttämaa

Laser-induced acoustic desorption (LIAD) enables the desorption of nonvolatile and/or thermally labile neutral compounds, such as asphaltenes, saturated hydrocarbons in base-oil fractions and biomolecules, from a metal surface into a mass spectrometer. This is a "gentle" evaporation technique and causes minimal fragmentation to the desorbed neutral molecules, including oligonucleotides and polypeptides. LIAD can be coupled with a wide range of ionization methods to facilitate analysis of the desorbed analytes by using many different types of mass spectrometers, including Fourier transform ion cyclotron resonance, linear quadrupole ion trap and quadrupole time-of-flight instruments. The development and improvement of LIAD remains an active research area with diverse goals such as better desorption efficiencies, minimized analyte fragmentation and greater versatility. This article details the theory, experimental methods, applications, and future directions of LIAD in combination with mass spectrometry.

Fundamental aspects

Introducing large, thermally labile compounds into the gas-phase environment of mass spectrometers without changing their structures has always been a challenge. To address this issue, various soft desorption/ionization techniques have been developed, such as matrix-assisted laser desorption ionization[1] (MALDI), electrospray ionization[2] (ESI), desorption electrospray ionization[3] (DESI), and laser-induced acoustic desorption[4] (LIAD) coupled with various ionization methods.[5,6] Among these techniques, LIAD is unique in that it desorbs neutral molecules instead of ions.

A typical LIAD device focuses a high-energy laser beam on the backside of a thin metal foil (typically ~10 μm). A thin layer of a solid sample is deposited on the front side of the foil. The laser irradiation causes desorption of intact, neutral sample molecules into the gas phase from the metal surface (if the conditions are such that thermal degradation of the sample does not take place). Many different ionization methods and mass analyzers have been utilized to ionize and analyze the gaseous molecules.

LIAD exhibits several major advantages over MALDI, ESI, and DESI, including the following:[6,7] (1) LIAD can be used to desorb large saturated hydrocarbons with no polar functional groups into the mass spectrometer for ionization, while these compounds cannot be desorbed using MALDI, ESI or DESI; (2) the translational energy and average velocity of the desorbed molecules are lower than for gas-phase ions generated using MALDI, thus allowing for more efficient ion–molecule reactions; and (3) sample desorption is decoupled from ionization, making LIAD a more versatile technique than MALDI, ESI, or DESI as many different ionization methods can be coupled with LIAD. However, the plume densities (10^9–10^{10} cm^3) generated upon some LIAD experiments have been reported to be less than those obtained upon MALDI, although still high enough for many experiments.[8] Due to these and other advantages, LIAD has become a common desorption technique, with its broadening field of applications summarized later in this article.

As for the working principle of LIAD, the exact mechanism(s) of LIAD is (are) still unknown, and different mechanisms may be operational under different experimental conditions. Substantial efforts have been made thus far to understand these mechanism(s), including both computational modeling and experimental measurements. Several models have been proposed and tested; the most important ones are discussed next.

Thermal waves

Although thermal waves are generated upon laser shots on a metal and may contribute to sample desorption, this is

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unlikely to be the mechanism of most LIAD experiments for the following reasons. First, as the translational velocity of the molecules after desorption is independent of the laser intensity that dictates the intensity of thermal waves, it has been claimed that thermal desorption is not occurring.[8] Second, the foil is selected to be so thick that there is no change in temperature on the sample side of the metal foil,[9] thus demonstrating that the sample is not desorbed due to thermal waves. Finally, labile compounds, such as oligonucleotides, desorbed with LIAD using 12-μm-thick Ti foils, undergo minimal to no fragmentation, which invalidates the presence of strong thermal waves.[10,11]

"Shake-off" mechanism

A "shake-off" model has been the most widely accepted description of the LIAD mechanism, yet it has its flaws. The presumptions are as follows: when a laser beam impacts the irradiation side of the metal foil, acoustic waves are generated and propagate through the foil, imparting energy to the sample molecules. The sample molecules obtain momentum normal to the sample surface and are thus ejected.[4,12]

The previously mentioned model is founded on a few well-established experiments. Pulsed laser beams with different energy densities have been demonstrated to interact with metal foils to cause different mechanical vibration responses.[13] When the irradiation side of the foil was placed against a glass surface and irradiated with a laser beam with an intensity greater than 10^8 W/cm,[2,14] a sharp mechanical wave was usually observed within 1 μs after the first laser pulse.[15] Therefore, the acoustic waves were hypothesized to create a sudden displacement of the foil surface, resulting in analyte desorption.

However, some observations have been reported to be in disagreement with this model. For example, the minimum required speed of foil displacement has been calculated[8] to be 100–500 m/s to desorb molecules with molecular weights of 200–500 Da from the surface. This range was concluded[9] to be in disagreement with the maximum 100 m/s speed that has been observed for LIAD experiments. Further, the observation that increasing the laser power intensity by two orders of magnitude had no effect on the measured velocities of the desorbed molecules[9] indicated that although acoustic waves were generated, they were not solely responsible for LIAD. However, it should be noted that the relationship between the laser power intensity and the velocities of the desorbed molecules is not fully understood at this time. Further, while the first and largest foil displacement has been reported to occur in less than 1 μs, desorption has been found to occur over a range of tens of μs.[16] Desorption due to "shake- off" was postulated to occur during the largest displacement, not over time.[16] These observations led to the introduction of the "film strain" mechanism that is discussed next.

"Film strain" mechanism

A new mechanism for LIAD was reported in 2007.[16] The basis of the mechanism was that the sample on the foil is not a continuous film, but crystals that experience "film strain." The molecules are liberated from the crystals due to surface deformation caused by a combination of thermal and acoustic waves. This may explain why the energy transfer to the molecules appears to be independent of laser power, as it would instead be derived from the stored energy of individual crystals.[16] However, the feasibility of this mechanism for the experiments involved in most analytical applications of LIAD is not clear because these experiments use a continuous sample layer on the foil, not crystals.

LIAD experimental setups

A typical LIAD setup consists of a laser with relevant optics, a substrate (usually metal foil) with a sample deposited on one side, and a means for positioning the system so that laser sampling can be performed on different spots of the foil. In the following section, different experimental setups for LIAD are discussed, followed by a discussion of several ionization methods that have been coupled with LIAD.

Desorption

The substrate onto which the sample is deposited is an essential part of a LIAD experiment. The best substrate is expected to absorb laser light, be capable of propagating acoustic waves, be thin enough to enable propagation of the acoustic waves to the sample side, and be thick enough to prevent thermal waves from reaching the sample during the desorption event.[10] Metal foils are often used because of their high thermal expansion coefficients, low thermal conductivities, and low reflectivity at certain wavelengths of light.[16] Considering these three properties, titanium excels compared to many other metals, including Ag, Al, Au, Cu, and Fe.[16] About 10-μm-thick titanium foils have been widely and successfully employed for LIAD of intact neutral molecules. Hg has even better properties, but is much more difficult to deal with experimentally.[17]

More recently, tantalum has been introduced as an alternative metal due to its much higher melting point (3290 K compared to 1941 K for titanium) as it can therefore withstand higher laser intensities.[7,18,19] However, consideration of the melting point may be irrelevant as no laser damage to titanium foils has been reported in the literature. Furthermore, research groups using tantalum have reported extensive fragmentation even to such simple and stable compounds as glycine. However, as these groups have employed high-energy methods (high-energy laser irradiation) to ionize the gas-phase molecules, it is difficult to determine whether the fragmentation was caused by LIAD or by ionization.[7,20] Another disadvantage of using tantalum includes the finding that molecules desorbed from tantalum move faster compared to those desorbed from titanium foil.[7]

Other than metal foils, aluminum-based thin-layer chromatography (TLC) plates have also been employed in LIAD experiments. The samples analyzed included mixtures of dye standards and drug standards. The samples were first separated using TLC plates that were composed of a 100-μm-thick

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layer of aluminum and a 200-μm-thick layer of gel. After separation, the plates were glued on microscopic glass slides by using glycerol. The glass slides were set on a stage that moved linearly at a speed of 0.01–0.02 cm/s. A pulsed infrared laser with a wavelength of 1064 nm and a power density of 2.1×10^9 W/cm^2 was employed to perform LIAD. The desorbed molecules were ionized by ESI and analyzed using an ion trap mass spectrometer.[9] Dye standards were deprotonated or doubly deprotonated under negative ion mode ESI, whereas drug standards were protonated under positive ion mode ESI. Most ions derived from TLC spots could be assigned to known mixture components. However, gel particles desorbed together with the analyte were a concern.[9]

Experiments carried out using copper foils with different thicknesses have demonstrated that a foil thickness of ~10 μm is optimal for LIAD experiments.[11] Thinner foils have been found to enable thermal waves to reach the analyte, thereby causing thermal degradation of the sample. On the other hand, thicker foils have been reported to result in less efficient desorption.[9–11]

Laser power density is another key factor controlling LIAD efficiency. Increasing laser power density from 5.4×10^8 to 9.0×10^8 W/cm^2 was found[16] to increase ion abundances. This also resulted in a shift in the distribution of the *m*/*z* (mass-to-charge ratio) values of ions derived from a polymer mixture to higher *m*/*z* values, as shown in **Figure 1**. These findings indicate that higher laser power densities result in more efficient desorption of larger molecules.

However, laser power density that is too high can be detrimental to the generation of acoustic waves via a shielding effect wherein the plume of metal ablated from the foil surface (when using high power density) attenuates the energy of the later laser pulses at the foil.[21] A high-power LIAD probe with kinematically mounted high-power reflective mirrors and a telecentric lens pair, shown in **Figure 2**, has been developed to decrease power loss from the beam path by reducing beam divergence.[22] Power loss of 75% in earlier experiments was reduced to 2% by using this device, resulting in better sensitivity and reproducibility.

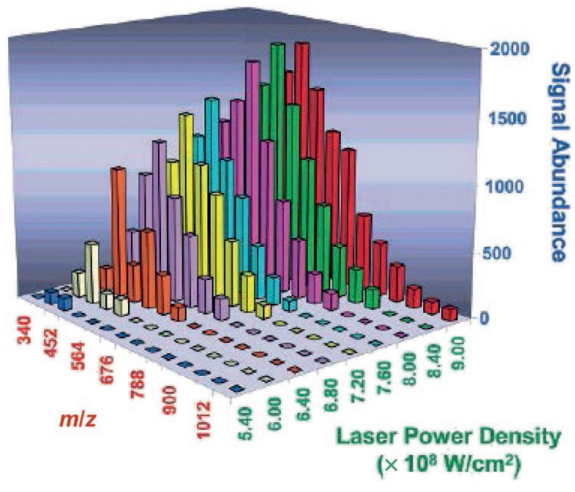


Figure 1. Relative ion abundances of polyisobutenyl succinic anhydride oligomers as a function of laser power density (different colors are used to make it easier to see which oligomers desorbed at each laser power density). Higher laser power densities result in more efficient desorption of larger molecules.[16] Note: *m*, mass; *z*, charge.

Analyte signal intensity in LIAD experiments relies heavily on the number of laser shots employed. However, each laser shot fired on a given spot of the foil depletes the sample at that spot. Therefore, the first few laser shots at any given foil location contribute the most to the amount of desorbed analyte. This phenomenon is illustrated in **Figure 3**.[16] Furthermore, firing many laser shots on a given spot on the foil may heat the sample. To increase the overall signal quality, a sampling mechanism can be employed that moves the laser to fresh spots on the sample foil or that moves the foil so that each laser shot is fired at a different spot. One way to do this is to rotate the sample foil while keeping the laser beam fixed, creating a circular sampling ring (**Figure 4**). The disadvantage is that this ring of sampled area is relatively small compared to the entire foil area and therefore most of the sample is not analyzed.[6,23] Improving on this method, a long rotating metal foil tape driven by a taper platform has been introduced to continuously provide new sample for a fixed laser beam, thus increasing the sampling area.[19] Yet another approach involves placing the sample foil on a stage that can be controlled in the *xy*-plane (Figure 4). This setup utilized nearly the entire sample area. Additionally, this setup made switching between analytes or samples easier because different samples could be deposited to different areas of the same piece of foil.[24]

Ionization

LIAD has been coupled with electron ionization (EI) and chemical ionization (CI) in Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers without external ion sources.[19,24,25] In these experiments, sample desorption and ionization took place in the cell (ion trap) inside the mass spectrometer under high vacuum (~10–^{9} Torr, 1.33×10^3 Pa). A LIAD probe loaded with the sample was positioned inside the vacuum chamber close to a trapping plate of the cell, and the laser was fired to desorb neutral analytes into the cell. This was followed by EI (electron kinetic energies of 60 eV or 70 eV) performed by firing an electron beam through the cell from a filament placed close to the cell. EI is a universal ionization method (all organic compounds are ionized) and highly reproducible, but can cause extensive fragmentation. The same LIAD setup was coupled with CI, a softer ionization method. This approach involved using the electron beam to ionize a reagent in order to generate CI reagent ions that were trapped in the cell, isolated, and then used to ionize the neutral analyte introduced by LIAD. A careful selection of the CI reagent ion can greatly reduce fragmentation. This approach, using protonated diethylaniline as the reagent ion, enabled LIAD and ionization of the tetrapeptide Val-Ala-Ala-Phe

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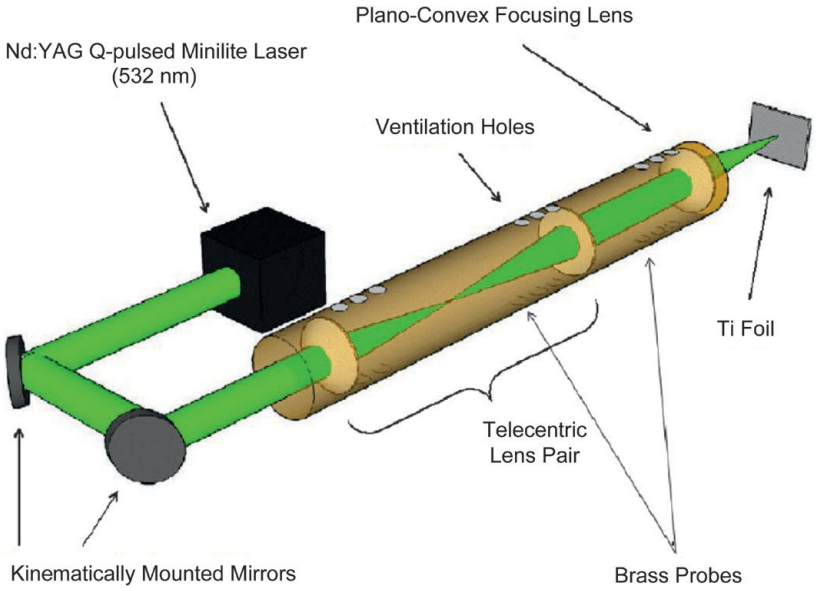


Figure 2. A high-throughput laser-induced acoustic desorption probe with kinematically mounted high-power reflective mirrors and a telecentric lens pair developed to increase power density by decreasing power loss from the beam path as well as reducing beam divergence.[22] Note: YAG, yttrium aluminum garnet (laser).

(VAAF, valyl alanyl alanyl phenylalanine containing the amino acids valine [Val], alanine [Ala] and phenylalanine [Phe]) with only minor fragmentation.[19]

The LIAD/CI experiment was later adapted to a linear quadrupole ion trap mass spectrometer.[26] A LIAD probe was inserted into the back of the instrument (**Figure 5**) to desorb neutral molecules into the ion trap. The desorbed molecules were then ionized by reagent ions that had been previously generated using ESI, transferred into the trap, isolated, and trapped. This method was demonstrated to be more sensitive for analysis of VAAF than the FT-ICR analysis previously discussed.[26]

LIAD has also been combined with tunable synchrotron vacuum ultraviolet photoionization (SVUVPI) in a quadrupole time-of-flight (QTOF) mass spectrometer.[27] Desorption and ionization of the analyte occurred in the vacuum chamber. Fragile compounds, including ergosterol (molecular weight [MW] 397 Da), were evaporated and ionized with little to no observed fragmentation by using the lowest laser energy that enabled ionization of the analyte.[27]

In addition to vacuum ionization methods, LIAD has been coupled with atmospheric pressure ionization methods. One of them is ESI. This is a soft ionization method typically used to characterize large polar compounds, such as proteins.[9,28,29] Different types of protein mixtures, such as protein standards, human serum, and cow's milk, were desorbed by LIAD, mixed into an ESI plume for ionization, and transferred into a QTOF mass spectrometer for analysis. Singly or multiply charged analyte ions of proteins were detected.[29]

Commercial atmospheric pressure chemical ionization (APCI) sources also have been coupled to LIAD.[22,30] APCI is beneficial compared to ESI as it can be used to ionize nonpolar as well as polar analytes. In these experiments, reagent ions (such as the radical cations of nitrogen and carbon disulfide [i.e., $N_2^{+\bullet}$ and $CS_2^{+\bullet}$]) were generated via APCI. Desorbed neutral analyte molecules were mixed with the plume of reagent ions and subsequently ionized.[22,30] Another approach involved introducing APCI reagent gas, O_2, through 16 small channels surrounding the sample foil and then mixing them with the

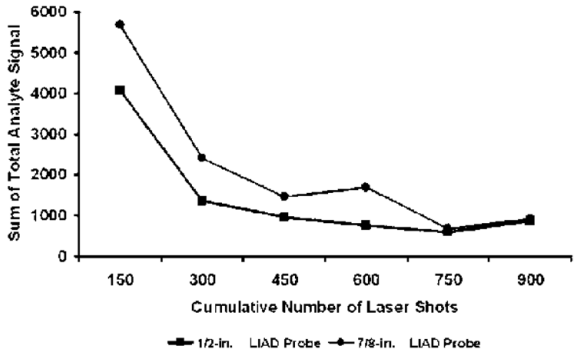


Figure 3. Sum of total analyte signal for a protonated tetrapeptide, valyl alanyl phenylalanine, as a function of cumulative number of laser shots fired on a circular sample foil. Two different laser-induced acoustic desorption (LIAD) probes, with a 1/2 in. and 7/8 in. dia., were employed. Each data point represents the total analyte signal obtained using $n \times 150$ laser shots (n = 1–6). The first 150 laser shots were each fired on a fresh spot on the foil, corresponding to one full rotation of the sample foil. The following set of 150 laser shots (data point at 300 total laser shots) were fired on the same spots that had already been sampled once, showing a decrease in analyte signal. After a total of about 750 laser shots (n = 5, meaning that each spot on the foil had been sampled five times), most of the analyte had been desorbed.[16]

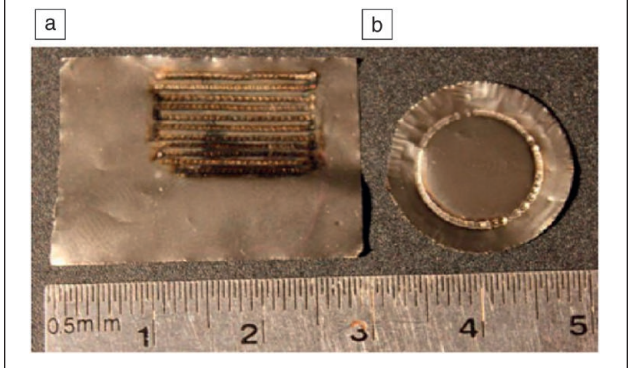


Figure 4. (a) A raster laser-induced acoustic desorption (LIAD) foil can be used to examine the analyte over a much larger area of the foil surface than (b) a circular LIAD foil (pictures taken after experiments).[22]

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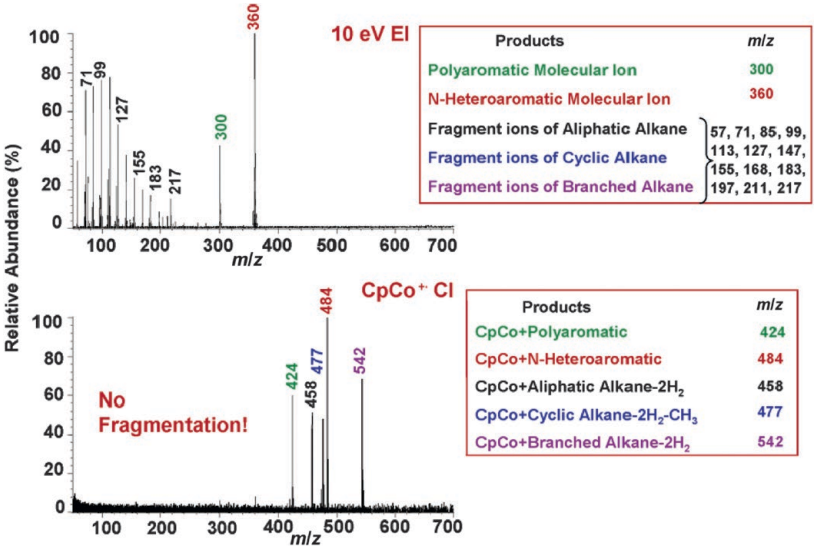


Figure 5. Comparison of laser-induced acoustic desorption/electron ionization (LIAD/EI) (top) and LIAD/$CpCo^{+\bullet}$ (bottom) mass spectra of a five-component model hydrocarbon mixture (~1:1:1:1:1:1; compounds are given in the red boxes).[40] The data demonstrate that only two components of the mixture yielded a stable molecular ion upon EI while all others fragmented extensively. However, use of the $CpCo^{+\bullet}$ reagent ion resulted in formation of a stable pseudo-molecular ion for all five compounds with no fragmentation. Note: Cp, cyclopentadienyl; *m*, mass of the ion; *z*, number of charges in the ion.

desorbed analytes.[31] The mixture was subsequently ionized by Corona discharge. This approach is referred to as atmospheric pressure laser-induced acoustic desorption chemical ionization.[31] Linear and branched alkanes and cycloalkanes were ionized to produce $[M—H]^+$ ions with minimal fragmentation.[31]

Atmospheric pressure photoionization (APPI) is an option for ionizing molecules with chromophores.[32] A vacuum ultraviolet lamp and toluene solvent have been utilized to ionize both aromatic and nonaromatic hydrocarbons desorbed via LIAD, including phenanthrene, perylene, and squalene.[33] Most analytes were ionized to give molecular ions with minimal fragmentation.

Applications and prospects

Applications in petroleomics

Many methods have been tested for the mass spectrometric analysis of heavy hydrocarbons (MW range 400–1500 Da) related to petroleomics (characterization of petroleum at the molecular level). These compounds are particularly challenging as they are not volatile, do not contain ionizable functionalities, and after ionization they often fragment extensively. For example, laser desorption ionization preferentially ionizes relatively small molecules[34] and suffers from aggregation and analyte fragmentation.[35,36] On the other hand, ESI can be used to evaporate and ionize only polar components of petroleum, while the majority of the components in petroleum are nonpolar hydrocarbons.[37–40] Field desorption/field ionization (FD/FI) is the method traditionally employed in the oil industry to desorb and ionize heavy hydrocarbon samples. This method is based on an emitter composed of needle-like whiskers onto which the sample is deposited.[41,42] High voltage applied to the whiskers causes desorption and ionization of the sample. However, FD/FI suffers from several problems, such as extensive ion fragmentation, poor reproducibility, mass bias, and variable sensitivity.[43–46]

LIAD/EI can be used to desorb and ionize heavy hydrocarbons in petroleum samples, but it often causes extensive fragmentation at high electron energies and has low ionization efficiency at low electron energies.[47] LIAD coupled to other ionization methods besides EI is a better option. LIAD has been coupled with CI in an FT-ICR mass spectrometer to evaporate and ionize large saturated hydrocarbons[48] and petroleum distillates with minor fragmentation.[40] In these studies, the cyclopentadienyl cobalt radical cation, $CpCo^{+\bullet}$, was utilized as the CI reagent ion. For the model compounds (R) and mixtures tested, the reagent ion formed an adduct ion $[R + CpCo]^{+\bullet}$ or fragment ions $[R + CpCo—H_2]^{+\bullet}$ or $[R + CpCo—2H_2]^{+\bullet}$ for each analyte, but in most cases, no further fragmentation was induced by this reagent ion. This method was tested for different types of compounds, including alkanes, alkenes, aromatic compounds, and heterocyclic compounds. Only limited fragmentation was observed. Therefore, this approach enabled the determination of the MW distributions for the analyzed mixtures.

This method also showed minimal ionization bias for different types of hydrocarbons (Figure 5). However, distinction of alkenes from analogous alkanes was challenging if the alkane produced $[R + CpCo—2H_2]^{+\bullet}$ as the major ions (Figure 5). Therefore, a better LIAD/CI approach was developed later.[47,49] This method employed the $ClMn(H_2O)^+$ reagent ion that only generated one product ion for each model compound (R) studied, the $[R + ClMn]^+$ ion, which is a benefit compared to the $CpCo^{+\bullet}$ reagent ion. The method showed minimal ionization bias toward different types of compounds in mixtures such as base oil samples. Further, analysis of saturated hydrocarbon polymers, such as polyethylene, was also successfully accomplished with this method and yielded results that agreed well with other techniques.[50]

LIAD coupled with APCI and using O_2 reagent gas (atmospheric pressure laser-induced acoustic desorption chemical ionization) in an FT-ICR mass spectrometer has been recommended for the analysis of large hydrocarbons, based on studies of model compound mixtures and a base oil sample.[31] The effects of N_2 and O_2 reagent gases on the ionization of the analytes were examined. When N_2 was used, molecular ions $M^{+\bullet}$ were formed for linear alkanes while $[M—H]^+$ ions were formed when O_2 was used. No fragmentation was observed in either case. Analogous ions were generated for branched alkanes. However, some

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model compounds, such as squalene (2,6,10,15,19,23-hexamethyltetracosane), experienced extensive fragmentation after ionization when using N_2 gas. Therefore, O_2 was concluded to be a better reagent gas. However, the use of O_2 resulted in the production of either $M^{+\bullet}$ or $[M–H]^+$ ions for cycloalkanes, depending on the temperature of the heated metal capillary. The MW distribution of the base oil sample as well as the elemental composition of each component in the sample were determined (**Figure 6**). These results were comparable to those obtained previously using LIAD/CI methods in an FT-ICR,[47,48,50] but the throughput of the experiment was higher (less than 3 min/sample versus ~30 min/sample). The analysis of the paraffin series in the base oil sample was in good agreement with the results obtained previously using FI/FD.[51]

Applications in studies of aromatic compounds

Gas-phase reactivity of the radical cations of polycyclic aromatic hydrocarbons (PAHs), such as naphthalene, anthracene, and phenanthrene, and their nitrogen-containing analogs (PANHs), such as quinoline, isoquinoline, and acridine, toward hydrogen atoms has been explored by using LIAD. These reactions are of interest for improving the understanding of the chemistry occurring for PAHs and PANHs in diffuse regions of interstellar medium, where both PAH and PANH radical cations and hydrogen atoms are abundant. In order to examine these reactions, LIAD was coupled with a flowing afterglow-selected ion flow tube instrument, a special type of a mass spectrometer designed for studies of gas-phase ion-molecule reactions at higher pressures than in most mass spectrometers.[52] LIAD enabled the introduction of the relatively large, nonvolatile PAH and PANH molecules into the flowing afterglow in sufficient concentrations for reactivity studies. LIAD/He^+ CI or Penning ionization using metastable Ar atoms was used to generate molecular ions from the gaseous molecules. Hydrogen atoms were generated by thermal dissociation of H_2 on a hot W filament. Addition of a hydrogen atom to all cations studied was observed. Interestingly, the reaction efficiencies of PAH and PANH cations with the same size and geometry (e.g., ionized anthracene versus acridine) were found to be identical.

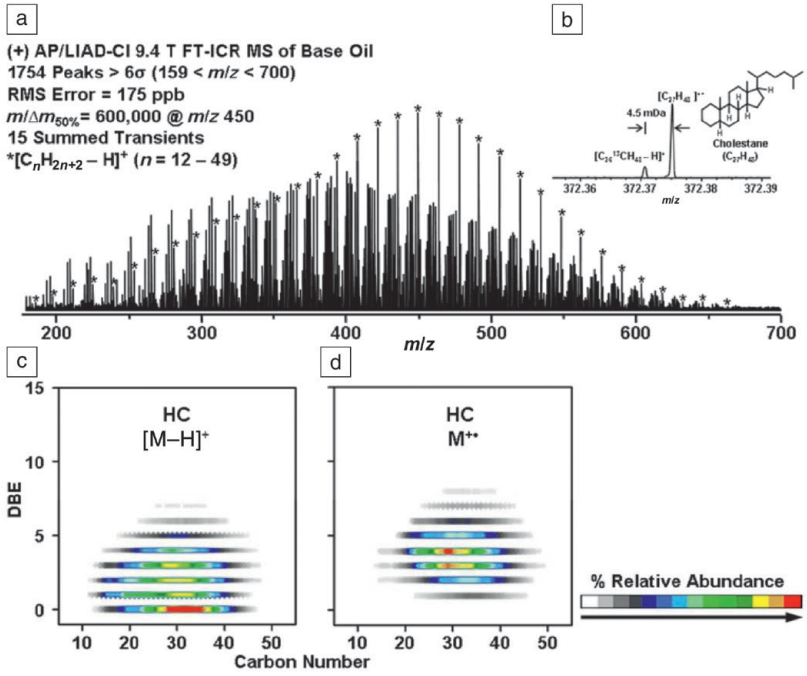


Figure 6. (a) Laser-induced acoustic desorption/atmospheric pressure chemical ionization (LIAD/APCI) 9.4 T Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrum for a base oil obtained using O_2 reagent gas. Reprinted with permission from Reference 31. © 2012 American Chemical Society. The most abundant peaks (highlighted by asterisks) correspond to a saturated hydrocarbon series. The mass-scale-expanded inset (b) highlights the requirement for ultrahigh resolution to separate ions that differ in elemental composition by ^{13}C versus CH (4.5 mDa difference in exact mass). (c, d) Isoabundance-contoured plot of double-bond equivalent (the number of double bonds and rings) versus the number of carbons for ions containing only carbons (C) and hydrogens (H). (c) The saturated hydrocarbon series (DBE = 0) corresponding to alkanes has the highest relative abundance for ions by hydride abstraction ($[M–H]^+$). The figure also shows higher DBE series corresponding to cycloalkanes with increasing number of rings (from one up to eight rings; DBE = 1–8). (d) When the heated metal capillary temperature was raised to ~250°C, cycloalkanes also generated molecular ions ($M^{+\bullet}$). Note: MS, mass spectrometry; RMS, root mean square; *m,* mass of the ion; $\Delta m_{50\%}$, mass spectral peak width at half maximum peak height; *z*, number of charges in the ion; DBE, double-bond equivalence; σ, standard deviation.

LIAD coupled to single-photon ionization ([SPI]; photon energy 7.9 eV) time-of-flight (TOF) mass spectrometer has been tested for the analysis of several organic dyes with low ionization energies.[15] Dominant molecular ions were generated for all of the compounds studied, though increased laser intensities led to increased fragmentation in some of the experiments. Later, LIAD was coupled to a tunable SVUVPI source and a QTOF mass spectrometer. The SVUVPI source provided photons with energies ranging from 7.8 to 24.0 eV, thus enabling ionization of a wider range of analytes. The technique was examined for the analysis of petroleum related aromatic compounds by using several different photon energies.[53] The extent of fragmentation was successfully controlled by selecting the lowest photon energy capable of ionizing the analytes. Interestingly, the mass distribution measured for one of the mixtures (**Figure 7**), composed of aromatic compounds and separated directly from Lungu atmospheric residue and not subjected any further treatment, was bimodal, peaking at 300 and 450 Da. The relative areas of these two peaks were found to change significantly with the photon energy (9.0, 11.0, or 14.0 eV), suggesting the presence of at least two categories of compounds with different ionization energies (i.e., polycyclic aromatic hydrocarbons with a molecular weight range of 400–900 Da

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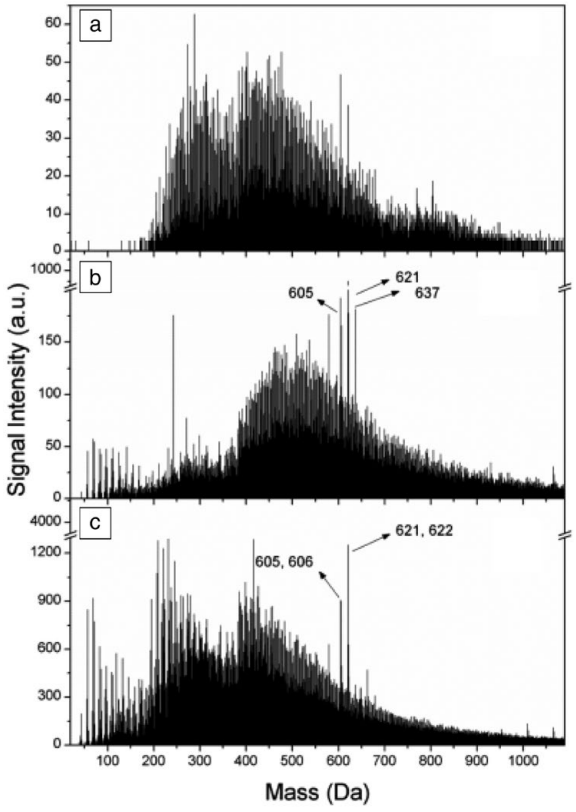


Figure 7. Laser-induced acoustic desorption/synchrotron vacuum ultraviolet photoionization mass spectra of a mixture of aromatic compounds separated from a Lungu atmospheric residue resin and measured using synchrotron vacuum ultraviolet photon energies of (a) 9.0 eV, (b) 11.0 eV, and (c) 14.0 eV. The relative areas of the major two peaks change significantly with the photon energy, suggesting the presence of at least two categories of compounds with different ionization energies. Adapted with permission from Reference 53. © 2013 American Chemical Society.

and aliphatic and alicyclic compounds with a mass range of 200–400 Da).[53]

Applications in life sciences

Mass spectrometric analysis of biopolymers, such as polypeptides and polynucleotides, is usually performed using MALDI or ESI to evaporate and gently ionize the molecules nearly simultaneously. However, in some instances, it is preferable to separate the evaporation and ionization steps. This is the case, for example, when ionization via reactions other than proton or metal cation attachment is desired. Furthermore, the ability to examine gas-phase reactions of net-neutral biopolymers with radical cations is sometimes desirable.[54] LIAD can be used to address both of these issues by vaporizing the intact neutral biomolecules into a mass spectrometer, followed by ionization using desirable reagent ions other than Brønsted acids, (i.e., reagents that do not protonate the biomolecules) or by exploring their gas-phase reactivity toward ionic reagents of interest.

Examination of LIAD/EI mass spectra of several amino acids revealed that this method generates similar mass spectra to those obtained using EI coupled with thermal heating to evaporate the amino acids.[23] Therefore, LIAD can be used to measure EI mass spectra for peptides and other compounds that cannot be evaporated using thermal heating. Indeed, this approach has been employed to measure EI mass spectra for several small peptides as well as their hydrochloride salts.[23] Further, LIAD/EI has been demonstrated to enable the differentiation of peptides containing leucine versus isoleucine.[23] A LIAD/CI method has been introduced for peptide sequencing.[55] Utilization of $P(OCH_3)_2^+$ as a CI reagent ion to ionize peptides desorbed by LIAD generated stable [peptide + P–2H]$^+$ ions. Subjecting these ions to collision-activated dissociation (CAD) yielded sequence-related product ions (**Figure 8**).

Further, LIAD has been utilized to desorb net-neutral nucleotides, dinucleoside phosphates, dipeptides, and tetrapeptides into the gas phase in order to study their reactions with charged phenyl radicals generated in an FT-ICR mass spectrometer.[56,57] The results were found to be consistent with those obtained for similar systems in aqueous solution.[58–61] Therefore, LIAD coupled with mass spectrometry (MS) provides a convenient method to study the fundamental reactivity of aromatic biradicals toward biomolecules, which is not straightforward when using solution experiments.

On the other hand, LIAD/CI in an FT-ICR mass spectrometer has been demonstrated to enable the detection of nonpolar lipids and steroid model compounds[24] ionized using $ClMn(H_2O)^+$ as the CI reagent ion. Little or no fragmentation was observed. These analytes either could not be ionized using APCI and ESI because they are nonpolar, or extensive fragmentation was observed (upon APCI).

The previously discussed LIAD/SVUVPI approach has also been used for the analysis of fragile compounds, such as ergosterol (a sterol found in fungi). The extent of fragmentation was controlled by selecting the lowest photon energy capable of ionizing the compounds.[27]

LIAD has been utilized to study the mechanisms of DNA strand breakage via dissociative electron attachment (DEA) of model compounds (thymidine and D-ribose-5-phosphate).[62] The neutral model compounds were desorbed by LIAD, then ionized and fragmented via DEA. When using electrons with near 0 eV kinetic energies, DEA caused the cleavage of the sugar–phosphate linkage in the d-ribose-5-phosphate, suggesting that DEA occurs directly in the DNA/RNA backbone.

Femtosecond pulsed lasers coupled to LIAD have been utilized to study extremely fast gas-phase dynamics of biomolecules, such as vibrational wave packet evolution.[63] As utilization of a femtosecond laser can provide information on interactions on time scales shorter than structural rearrangements and charge-transfer processes, it may be more suitable for peptide sequencing and for causing selective bond cleavages than the use of nanosecond lasers.[18,64]

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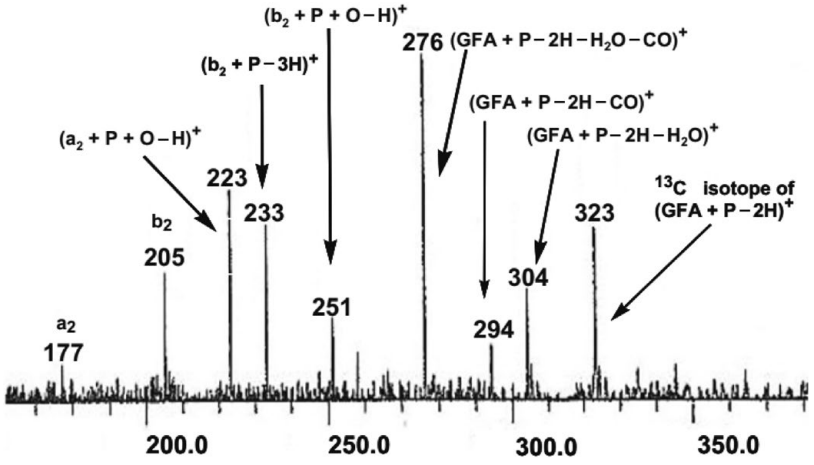


Figure 8. A collision-activated dissociation mass spectrum of [GFA + P–2H]+ ions (GFA or gly-phe-ala is a tripeptide containing the amino acids glycine, phenylalanine, and alanine) obtained to study peptide sequencing. The various sequence-related fragment ions are indicated.[55] Note: a_2 and b_2 are symbols for common peptide fragment ions.

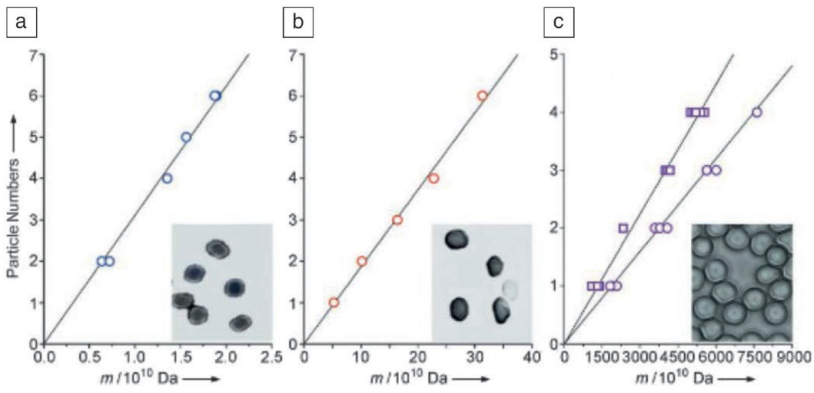


Figure 9. Plots of the number of particles as a function of their mass (m) measured for (a) vaccinia viruses (inset: electron microscope image), (b) *E. coli* K-12 bacteria (inset: electron microscope image), and (c) human red blood cells (inset: optical microscope image). The two data sets shown for (c) are those of normal (○) and anemic (□) red blood cells. As cluster particles are formed during the laser desorption process, least-squares fitting of measured masses versus particle numbers was used to derive the mass of the monomeric particles. Adapted with permission from Reference 65. © 2006 Wiley.

Fragmentation of the ionized analytes could be controlled by changing the pulse length of the laser.[63]

LIAD has been successfully used to desorb and detect vaccinia viruses, *E. coli* K-12 bacteria, red blood cells, and other bioparticles in a three-dimensional quadrupole ion trap mass spectrometer.[65] These bioparticles were charged, and therefore, no external ionization was needed in this experiment (**Figure 9**).[65] The m/z value of each single particle was determined by observing the light-scattering pattern, which is related to the trap-driving frequency ($\Omega/2\pi$) and voltage amplitude (V_{ac}), when using a charge-coupled device for collection of scattered photons. The feasibility of this method was demonstrated by measuring the sizes of polystyrene particle standards.[66–68]

Prospects

LIAD has thus far been demonstrated to enable the evaporation of a variety of nonvolatile and thermally fragile neutral compounds into mass spectrometers, ranging from large nonpolar compounds, such as large saturated hydrocarbons, to fragile polar biopolymers, such as oligonucleotides. LIAD has even been used to desorb charged particles, such as bacteria. The gas-phase molecules have low internal and kinetic energies when proper experimental conditions are employed. They can be ionized using a wide variety of methods (EI, CI, SPI, ESI, APCI, APPI, and Penning ionization), which sets this method apart from such commonly used evaporation/ionization methods as MALDI, ESI, and DESI. The ability to use almost any ionization method in combination with LIAD enables unprecedented control over ion energetics. LIAD can be operated in high vacuum and at atmospheric pressure and anywhere in between, and it can be coupled with most mass spectrometers, making this method quite versatile.

Future applications of LIAD/ MS will be diverse. For example, LIAD/MS is anticipated to lead to improvements in disease diagnostics; it has been used already to distinguish normal red blood cells from anemic red blood cells.[65] Coupling of LIAD with new types of mass spectrometric methods, such as ion mobility MS, will enable more detailed studies of large gas-phase molecules and ions and will facilitate identification of isomers that present a challenge for other mass spectrometric methods.[19] The efficiency of desorption will be increased and the extent of ion fragmentation will be decreased, thus making LIAD/MS even more powerful than it is today.

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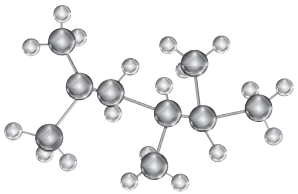


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