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# Laser Desorption-Atmospheric Pressure Chemical Ionization Mass Spectrometry for the Analysis of Peptides from Aqueous Solutions

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**A recently reported ionization method, comprising an infrared (IR) laser pulse to desorb (LD) analyte species, followed by atmospheric pressure chemical ionization (APCI) with a corona discharge (LD-APCI) to effect ionization of the desorbed neutral analyte molecules, is described for the direct analysis of aqueous peptide solutions. The source employs a heated capillary atmospheric pressure (AP) inlet coupled to a quadrupole ion trap mass spectrometer and allows sampling under normal ambient air conditions. By use of the corona discharge, signals of the atmospheric pressure infrared matrix-assisted laser desorption/ionization (AP-IR-MALDI)-generated analyte protonated molecule were enhanced by factors as large as 1400. In addition, the acid modifier trifluoroacetic acid (TFA) was found to improve the AP-IR-MALDI-generated signal by a factor of ∼10, whereas the LD-APCI generated signal yielded a 100-fold increase. In this study, the use of the corona discharge is described to enhance the analyte signal generated via AP-IR-MALDI and, as a tool, to probe the gas-phase neutral molecule population generated by the MALDI process. Finally, through the decoupling of desorption from ionization, implications regarding the application of LD-APCI for the direct analysis of numerous new analyte containing matrixes (e.g., polyacrylamide gel electrophoresis (PAGE), tissue, etc.) are discussed.**

Of late, a new method for performing matrix-assisted laser desorption/ionization (MALDI) at atmospheric pressure (AP) has been introduced as AP-MALDI.[1] For this technique, the heated capillary inlet of a Finnigan LCQ ion trap instrument was utilized to transport AP-MALDI-generated ions into vacuum for mass analysis.[2−4] More recently, an infrared (IR) laser at 3.0 $\mu$m was used to perform AP-IR-MALDI of peptides directly from aqueous solutions.[5]

Taking a different approach, we have used an IR laser at 10.6 $\mu$m to effect the desorption of neutral molecules at AP, followed by ionization in the gas phase with a corona discharge (LD-APCI).[6,7] By separating the desorption and ionization processes, the signal of the AP-IR-MALDI-generated spectra was enhanced 150-fold.[7] Kolaitis and Lubman, recognizing these advantages, reported in 1986 the first chemical ionization of laser-desorbed neutral molecules at AP using a $^{63}$Ni atmospheric pressure ion source.[8] Our work builds on that study through the use of a corona discharge, a more efficient ionization method, and via laser desorption in the IR, as opposed to the ultraviolet (UV) used in that work. This technique draws on the fact that a substantially larger number of gas-phase neutral molecules, as opposed to ions, is produced during a desorption event.[9] Moreover, the corona discharge is known to provide efficient ionization, and because it operates at AP, the process is quite gentle.

There are several advantages driving the development of AP laser desorption/ionization. First, AP-generated ions are more efficiently thermalized than those produced under vacuum conditions, producing spectra showing less fragmentation.[1] Second, AP sampling allows for the examination of vacuum-sensitive samples, such as tissue, without adverse effects and permits the exploration of new matrixes, such as liquid water. Third, restrictions of laser-focusing optics, imposed by the vacuum system, are removed at AP, thereby allowing use of near-field optical probes that can greatly reduce laser spot size.[10] Finally, instruments possessing atmospheric pressure inlets would be compatible with a variety of ionization sources (electrospray (ESI), APCI, AP-MALDI, LD-APCI).

The notion of decoupling desorption from ionization is not without precedence. For instance, Cotter and co-workers reported that a significant number of neutral molecules were produced as a result of a laser desorption event and that these neutral molecules had a longer lifetime in an ion source.[9,11] Specifically, he demonstrated that laser desorption ions were generated for a period of ∼1 $\mu$s, whereas neutral molecules were observed for

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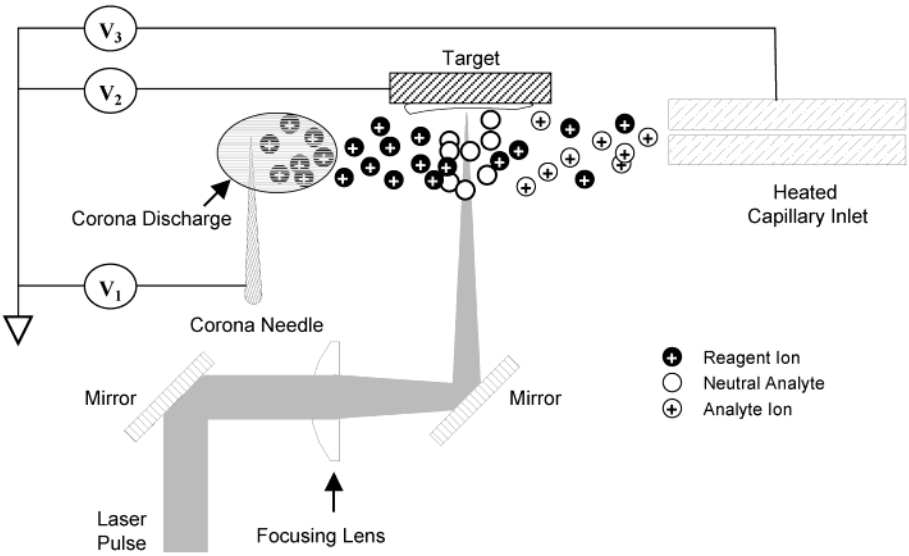


Figure 1. Schematic representation of LD-APCI source.

hundreds of microseconds. To exploit these desorbed neutral molecules, Cotter performed laser desorption in a chemical ionization source (LD/CI, $P \sim 0.5$ Torr) where the desorbed neutral molecules were ionized with reagent ions from a chemical ionization gas.[11]

In the following years, several different approaches were used to ionize the population of neutral molecules produced after a laser desorption event. These methods included electron impact ionization,[9] chemical ionization,[11−13] and resonant multiphoton ionization.[14−17] Even though the ionization method in each of these cases was different, the idea was the same: decoupling of the desorption and the ionization processes allows for the individual optimization of the two steps with increased efficiency and selectivity.

Despite the advantages that came from the decoupling of laser desorption from the ionization processes, these techniques were successful in desorbing only a few relatively small peptides. The desorption event, which often induced fragmentation and decomposition, was the limiting factor for the production of gas-phase neutral biomolecules and peptides. This limitation was overcome with the discovery that by using an absorbing, acidic matrix, biomolecules and peptides contained within that matrix could be desorbed/ionized (MALDI), producing intact molecular ions with little fragmentation.[18,19] Since that time, MALDI has become one of the most important ionization methods for biomolecules.

As a consequence of this success, the concept of decoupling desorption from ionization has received little attention over the past decade. Even so, Speir and Amster utilized a common MALDI matrix as a substrate for the peptide gramicidin S to demonstrate the functionality of LD/CI.[20] In more recent work, Belov et al. enhanced the MALDI signal of gramicidin S by reacting the produced gas-phase neutral peptide molecules with $Na^+$ ions.[21]

We describe here the use of an IR laser pulse to desorb gas-phase neutral peptide molecules at AP, followed by ionization in the gas phase using a corona discharge. In this orientation, the corona discharge can assume a variety of roles. In this paper, two of these roles are examined: (1) to provide a means for the gas-phase ionization of laser-desorbed peptide molecules and (2) to probe the AP-IR-MALDI process, exploring the efficiency of common MALDI modifiers, such as trifluoroacetic acid (TFA).

Other significant advantages could be gained from decoupling desorption from ionization. In MALDI, matrixes must not only assist with the transport of the analyte into the gas-phase, but must also provide a means for ionization. However, in LD-APCI, the matrix containing the analyte need not assist with the ionization, thereby opening the door to many new possible analyte-containing matrixes, including biological solutions, tissues,[22−24] polyacrylamide gels,[25,26] and thin-layer chromatography plates.

## EXPERIMENTAL SECTION

The LD-APCI source interface is presented in Figure 1, with a more detailed description found elsewhere.[7] Briefly, this design utilizes a heated capillary, AP inlet (ThermoFinnigan, San Jose, CA) to transport the AP generated ions into vacuum for mass analysis. Samples were applied directly to a 4-mm diameter stainless steel removable target that was located ~3 mm axially from the heated capillary inlet and ~2 mm offset from center. The target was held at an offset potential ($V_2$) of +2 kV (model 205A, Bertan Associates, Hicksville, NY). The corona needle was positioned ~3 cm from the inlet of the heated capillary with the tip axially aligned. A potential of +8.1 kV ($V_1$), from a standard

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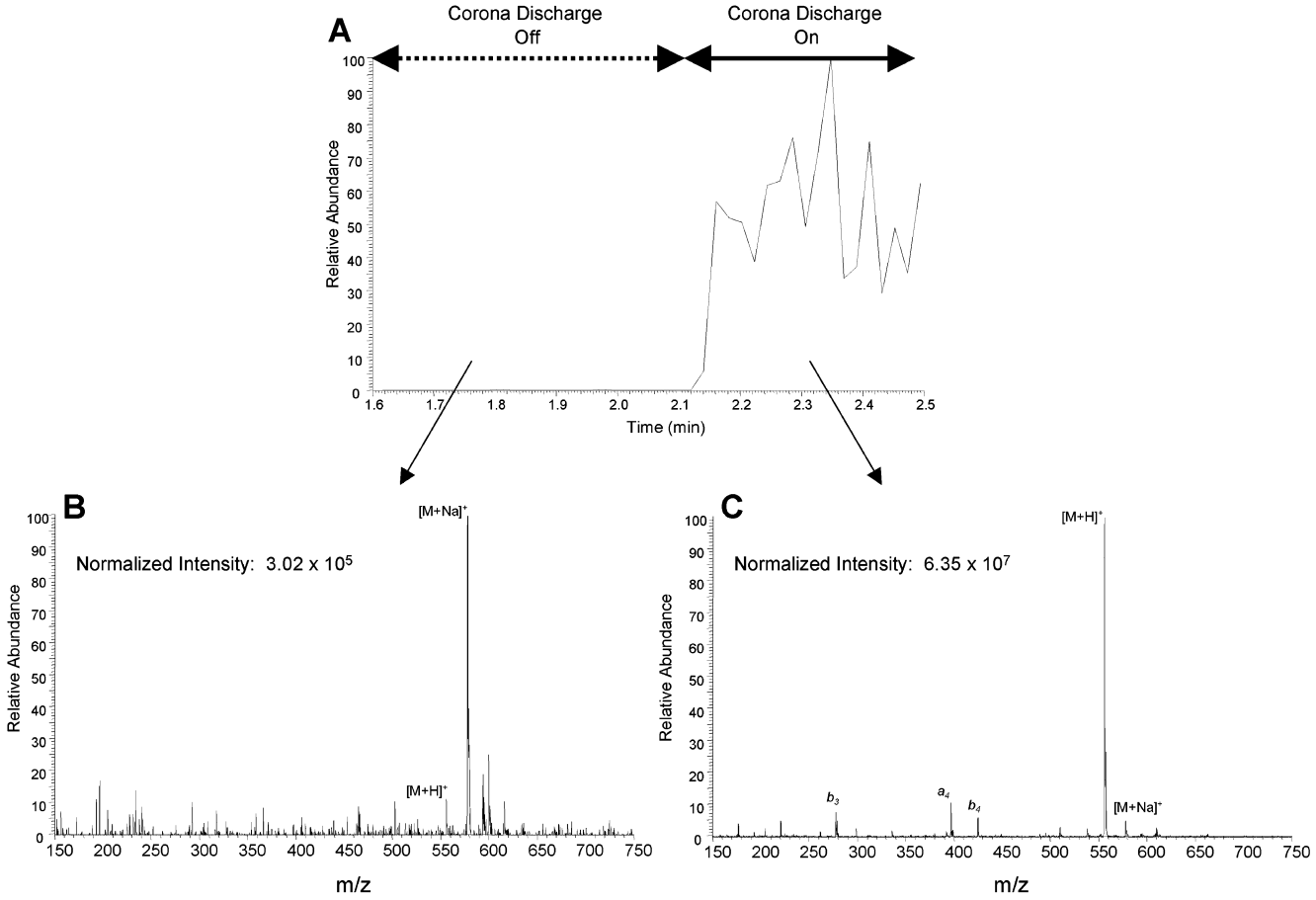


Figure 2. LD-APCI of leu-enkephalin in 50% aqueous glycerol solution (100 pmol loaded): (A) ion chromatogram for $[M + H]^+$ of leu-enkephalin (*m*/*z* 556) during constant scanning with corona discharge toggled off and on, (B) mass spectrum with corona discharge off (100 single-shot spectra averaged), and (C) mass spectrum with corona discharge on (5 single-shot spectra averaged).

ESI power supply (Analytica, Branford, MA) was used to generate the corona discharge.

Laser desorption was achieved by irradiation of the target with a pulsed $CO_2$ laser operating at 10.6 $\mu$m ($\mu$-TEA, Laser Science Inc., Franklin, MA). The beam was focused to a spot diameter of ~1.5 mm (~$2.2 \times 10^6$ W/cm$^2$, assuming homogeneous distribution) using a 10-cm focal length zinc selenide lens (Laser Research Optics, Providence, RI). The laser pulsing was synchronized to coincide with the prescan period of the scan function, which was 1 ms before the ion injection period of each microscan.

The mass spectrometer used in these studies, a modified quadrupole ion trap system (Finnigan GCQ, ThermoFinnigan, Austin, TX), was adapted to accept a two-stage differentially pumped vacuum chamber and was fitted with an Analytica ESI source manifold (Branford, MA).[7,27] This manifold was further modified to accept a metal heated capillary AP inlet. To prevent solvation of the ions, the heated capillary was maintained at 200 °C for the work presented here; an offset potential ($V_3$) of +130 V was applied to assist declustering.

The peptides leu-enkephalin, neurotensin 8−13, des-Pro$^2$ bradykinin, bradykinin, vasopressin, and angiotensin I (Sigma, St. Louis, MO) were dissolved in water (HPLC grade, Fisher Scientific, Fair Lawn, NJ) at a concentration of 1 mg/mL. For sample preparation, an aliquot of each solution was mixed with an equal amount of glycerol (Fisher Scientific, Fair Lawn, NJ) and vortexed to ensure homogeneity. A small amount of the mixture was deposited onto the surface of the target for sampling (typically 0.15−0.25 $\mu$L). In some cases, the peptide containing aqueous glycerol solution was dosed with 0.1% TFA. In spectra that portray 10 pmol loaded on target, the initial aqueous solution was diluted 10-fold, then mixed with glycerol in equal proportions as described above. By using liquid matrixes, such as aqueous glycerol, the target stage did not require *x*−*y* manipulation, and despite the small volumes applied to the target, the analyte signal could typically be generated for a continuous 10-min period.

## RESULTS AND DISCUSSION

In the past few years, several research groups have reported the use of aqueous glycerol solutions for IR-MALDI experiments, some using lasers emitting at wavelengths around 3 $\mu$m,[28,29] and others at 10.6 $\mu$m.[30] In those studies, the water was evaporated or frozen before introduction into the vacuum system of the mass spectrometer. More recently, aqueous glycerol solutions, used without freezing or evaporation, have been successfully employed

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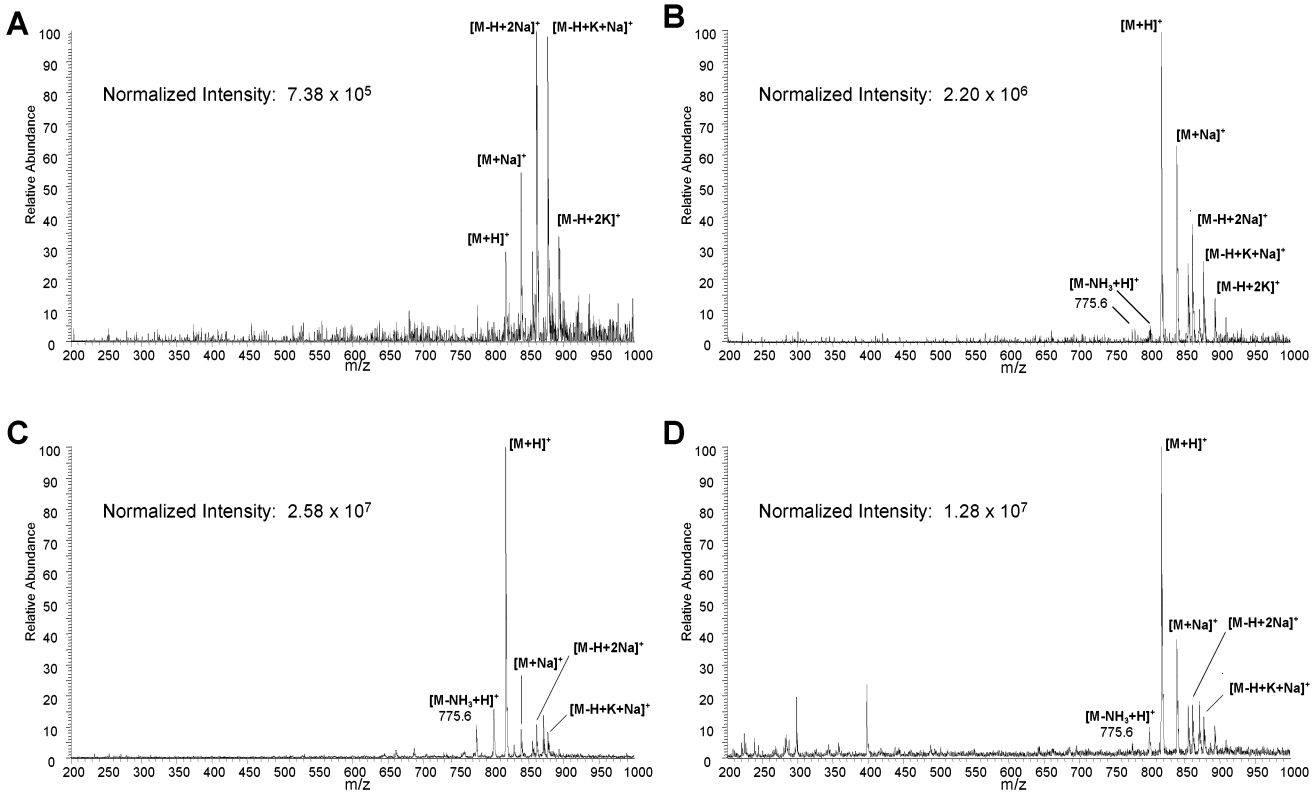


Figure 3. A and B represent AP-IR-MALDI generated spectra: (A) without 0.1% TFA, (B) with 0.1% TFA. C and D represent LD-APCI-generated spectra: (C) without 0.1% TFA, (D) with 0.1% TFA. The sample consisted of 100 pmol of neurotensin fragment 8−13 in a solution of 50% aqueous glycerol, with each spectrum representing the average of 25 single-shot spectra.

for AP-IR-MALDI of peptides with a laser emitting at 3 $\mu$m.[5] On the basis of the success of these collective works, we elected to utilize aqueous glycerol solutions of peptides to study the LD-APCI process for peptides and to provide a direct comparison to AP-IR-MALDI.

**Comparison of AP-IR-MALDI and LD-APCI.** Our initial studies were to determine any benefit gained through the use of the corona discharge, and for this evaluation, the pentapeptide leu-enkephalin was chosen. The effect of the corona discharge can be observed in Figure 2A, which displays the ion chromatogram of the $[M + H]^+$ ion of leu-enkephalin at $m/z$ 556, with the corona discharge turned off and on during continuous scanning. Figure 2B andC exhibits mass spectra that were obtained without and with the corona discharge, respectively. The spectrum presented in Figure 2B represents the average of 100 single-shot mass spectra (required to generate a quality spectrum), whereas Figure 2C represents the average of only 5 single-shot mass spectra.

Some significant differences can be observed comparing Figure 2B and C. Perhaps most distinguishing is the pronounced $[M + Na]^+$ peak observed under AP-IR-MALDI conditions (corona discharge off), while the $[M + H]^+$ dominates the LD-APCI spectrum (corona discharge on) with an enhancement by a factor of ∼1400. This demonstrates that under the AP-IR-MALDI conditions, cationization is the dominating ionization process. In contrast, the initiation of the corona discharge establishes an alternative means of ionization, namely, gas-phase proton transfer explaining the substantial enhancement of the protonated molecule that is observed.

Another noteworthy observation is that the absolute intensity of the sodiated molecular ion increases by an order of magnitude with the use of the corona discharge. One plausible explanation is that as a consequence of laser irradiation, the sodiated neutral peptide molecule $[M - H + Na]^0$ is liberated into the gas phase. By use of the corona discharge, this minority species can undergo gas-phase protonation, resulting in an increased formation of the $[M + Na]^+$.

Finally, the $b_4$, $b_3$, and $a_4$ series fragment ions of leu-enkephalin are easily observed in Figure 2C, but can also be seen (with larger amounts loaded, data not shown) without the use of the corona discharge. Because the fragmentation can be observed under both ionization conditions, the likely cause can be attributed to collisional-induced dissociation (CID) in the region between the heated capillary exit and skimmer cone. With our home-built source, a high offset potential of ∼ +250 V must be applied to a tube lens (between the heated capillary exit and the skimmer cone) to assist declustering and to provide for a more efficient transmission of higher mass ions.[7] For leu-enkephalin, the fragmentation could be somewhat relieved by lowering this offset, but the signal of the molecular ion was also reduced. Further supporting this theory, the fragmentation was most pronounced with the lower mass peptides studied (e.g., leu-enkephalin), those that would be accelerated the most by the offset voltage.

**Evaluation of the Acid Modifier TFA.** Acid modifiers, such as TFA, are often used during MALDI matrix solution preparation[31] and have been reported to be advantageous in some cases.[32] In prior AP-IR-MALDI experiments conducted by Laiko et al., 0.1%

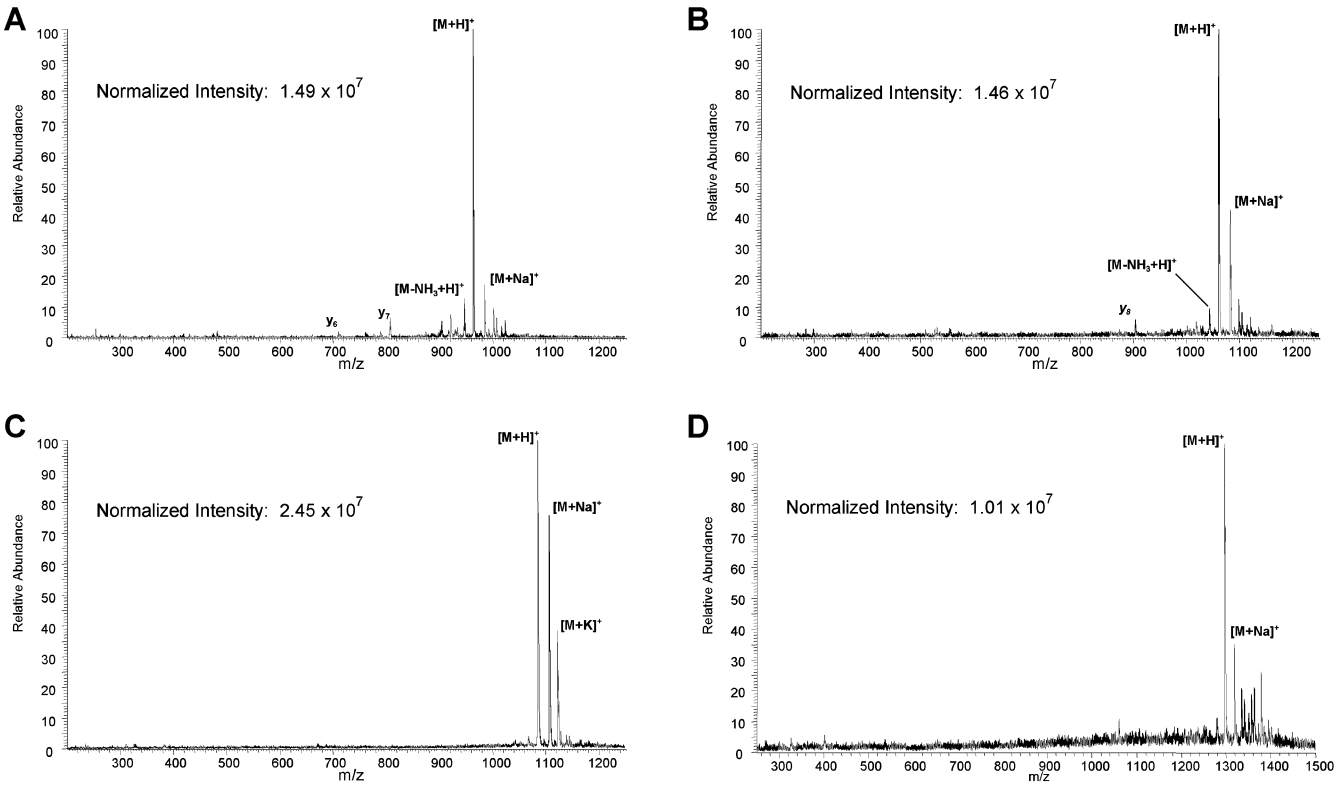


Figure 4. LD-APCI mass spectra of (A) des-Pro$^2$ bradykinin, (B) bradykinin, (C) arg-vasopressin, and (D) angiotensin I. Each spectrum represents the average of 25 single-shot mass spectra with 100 pmol of analyte loaded.

TFA was added to the matrix solutions, but no comments were made regarding the role or effect of the acid.[5]

Figure 3 displays a series of mass spectra obtained from a 50% aqueous glycerol solution containing the peptide neurotensin 8−13 both without (Figures 3A,C) and with (Figure 3B,D) 0.1% TFA. The effect of TFA on the AP-IR-MALDI-generated spectra can be seen by comparing Figure 3A and B. Here, the production of the protonated molecule ($m/z$ 817) is enhanced by a factor of ∼10 through the use of the TFA additive. In this case, the TFA, like the corona discharge, is providing an alternate ionization route, that of protonation, as opposed to cationization. Figure 3C,D displays the effect of the corona discharge for the solution sampled in Figure 3C, which does not contain TFA, while Figure 3D does. Using the corona discharge to enhance the AP-IR-MALDI generated signal provides a ∼100 fold increase in protonated molecule production without the TFA additive, a ∼10-fold enhancement over the TFA additive.

Figure 3D displays the LD-APCI spectrum of the TFA containing matrix. Here, the signal of the protonated molecule is somewhat suppressed, as compared to the signal generated with no TFA present, but perhaps more distinguishing is the elevated background. Specifically, a number of low-mass species can now be observed that were not previously present. These ions were also detected in a blank, and we propose that their formation is due to an excess of reagent ions formed by the initiation of the corona discharge. When TFA is present, a larger number of the analyte molecules are ionized, as evidenced by comparing Figure 3A and B. Consequently, the population of gas-phase neutral analyte species is lowered, so when the corona discharge is initiated, the excess reagent ions will participate in ionization of the lower proton affinity matrix and background molecules.

To summarize, these data show that for AP-IR-MALDI at 10.6 $\mu$m, TFA enhances generation of the protonated molecule. However, also demonstrated is that the use of a corona discharge can further enhance that signal by an order of magnitude. Because TFA is corrosive and its simultaneous use with the corona discharge was found to elevate background, the remaining work presented here was conducted without its use.

**LD-APCI of Various Peptides.** Figure 4A−D displays the LD-APCI-generated spectra of several other peptides. The peptides sampled include des-Pro$^2$ bradykinin (Figure 4A), bradykinin (Figure 4B), arg-vasopressin (Figure 4C), and angiotensin I (Figure 4D). In each case, the spectrum presented represents the average of 25 single-shot spectra with 100 pmol of each peptide loaded. The enhancements observed with the corona discharge were similar to those previously described in Figure 2. In fact, the equivalent AP-IR-MALDI spectra for angiotensin I failed to produce observable protonated molecules above the noise, and cationized adducts were present at low signal-to-noise (∼2). Therefore, despite the relatively high background that can be observed in Figure 4D, the LD-APCI method produced a marked improvement, as compared to AP-IR-MALDI.

**Sensitivity Considerations.** For an analytical technique, a measure of potential usefulness can be made by an evaluation of sensitivity. To assist this evaluation, a 10-fold dilution of the

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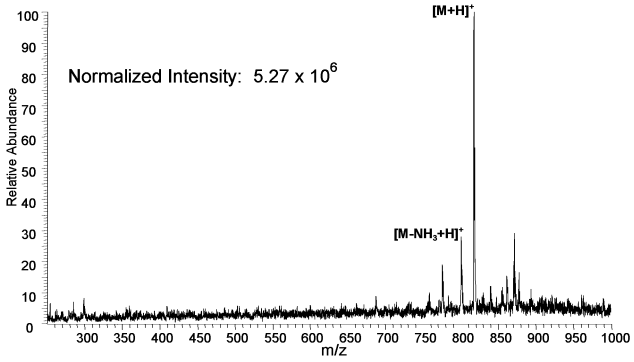


Figure 5. LD-APCI mass spectrum of neurotensin 8−13 (spectrum represents the average of 50 single-shot mass spectra, 10 pmol loaded).

neurotensin 8−13 containing aqueous glycerol solution was performed and analyzed. Figure 5 displays the spectrum generated after the deposition of this solution onto the target (10 pmol loaded, average of 50 single-shot spectra). Again, an intense protonated molecule was observed, but the background was elevated as a result of the dilution. At this level the corona discharge still induced an enhancement similar to those outlined above (data not shown). Next, the sample was diluted by another factor of 10 and analyzed, but no analyte ions could be observed.

Several parameters could enhance the sensitivity of the current LD-APCI methodology. To date, most AP-MALDI experiments have used multiple laser pulses per an extended ion accumulation period (200−400 ms)[2,4,5] to increase sensitivity, a method that is in sharp contrast to the single laser pulse followed by an ion accumulation period of ∼20 ms used in this study. The incorporation of multiple laser pulses could potentially be advantageous for LD-APCI to increase sensitivity. Other important considerations are the position of the corona needle and target and magnitude of the potential supplied to each, parameters that upon further optimization are expected to improve ionization efficiency and transport. An alteration of reagent gas makeup (presently ambient air) should also show an effect upon the ionization efficiency. Pulsing the corona discharge, time-delayed appropriately after each laser pulse, could add advantageous temporal resolution and preferential discrimination among ion signals.[33] And not to be underestimated, the interfacing of this source to a modern, commercial instrument could provide significant improvements as well.

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## CONCLUSIONS

This work demonstrates that a corona discharge enhances the production of protonated molecules of numerous peptides following laser desorption at AP. In addition, the acid modifier TFA was found to increase the production of the protonated molecule by a factor of ∼10 under AP-IR-MALDI conditions. The corona discharge enhances the protonated molecule signal by a factor of ∼100. From these data, it is clear that the ionization efficiency of the AP-IR-MALDI process can be significantly improved through the use of a supplemental ionization method, such as the corona discharge.

Because the LD-APCI process does not require the matrix to play a role in ionization, numerous new analyte-containing matrixes can potentially be compatible with this method. Some examples might include the direct analysis or imaging of biological tissues or solutions (e.g., blood plasma or urine) at atmospheric pressure. Although matrix extension is an important advantage that is possible with LD-APCI, the corona discharge can also be used as a valuable tool to examine the MALDI process. In short, it can be used as a probe to explore the gas-phase neutral population produced in the MALDI plume.

Finally, we have examined the LD-APCI sensitivity, which even in its present prototype form is reasonably good, but further improvements should result from thorough parametric optimization studies, including the use of multiple laser pulses per ion accumulation period, target and corona needle positioning and voltages, and reagent gas composition alterations.

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