

Direct Access to Isolated Biomolecules under Ambient Conditions**

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The study of large molecular systems in the gas phase has received a tremendous boost by the introduction of soft ionization methods for mass spectrometry (MS), in particular electrospray ionization (ESI),^[1] matrix-assisted laser desorption/ionization (MALDI),^[2] and related ambient ionization techniques.^[3] Quite heterogeneous populations of species, including abundant charged and neutral droplets/clusters of different sizes and compositions,^[4] are created by ESI and MALDI. The eventual formation of isolated molecular ions is believed to be linked to their observation by MS: the detection in vacuum effectively completes the desolvation and declustering processes.

In recent years, attention has shifted from merely observing intact gas-phase ions by MS to studying their structure and conformation,^[5] which generally requires much more sophisticated instrumentation, including but not limited to the combination of MS with ultraviolet,^[6] infrared multiphoton,^[7] UV/IR double resonance,^[8] and visible-light^[9,10] optical spectroscopy of ions trapped in the high vacuum of a mass spectrometer. The necessity for ion trapping, transport, and sometimes manipulation renders such instruments expensive and difficult to operate. Furthermore, direct spectroscopic interrogation is often not possible because of the low density of ions produced, which is dictated by the limited trapping capacity and ion-guide efficiency of the associated MS equipment. Instead, one typically has to resort to “action spectroscopy”, that is, the observation of fragment ions produced by optical excitation. Access to isolated nonvolatiles already at ambient conditions, without the need for complex instrumentation, would thus be highly advantageous.

Herein we unambiguously show that ESI is capable of generating high numbers of gaseous analytes at ambient

conditions. This was demonstrated by correlating fluorescence spectra recorded in the ESI plume with those of isolated ions in the high vacuum of a mass spectrometer. Therefore, isolated biomolecular ions are now directly accessible for spectroscopic investigation at ambient conditions, without the need for ion trapping and transport.

An experimental setup for the optical profiling of an ESI plume by laser-induced fluorescence (LIF) is shown schematically in Figure 1 a. We found that the detected fluorescence changes dramatically when different regions of the plume are

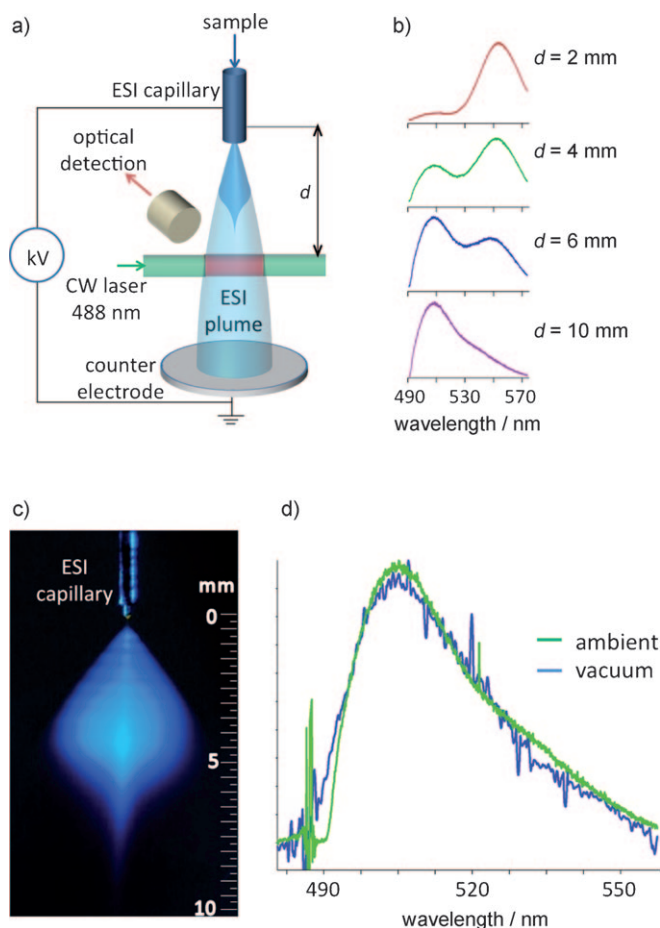


Figure 1. a) Experimental setup for optical spectroscopy in an ESI plume. b) Fluorescence spectra obtained from different regions in the ESI plume when a solution of R6G in methanol was electrosprayed. c) A photograph of the upper region of the ESI plume that was taken when the excitation laser beam was unfocused so that the whole plume area was illuminated; the blue color is due to laser light scattering from droplets in the plume. d) Fluorescence spectra of R6G ions recorded by FTICR MS–LIF spectroscopy (blue) and in the ESI plume in (b), bottom (green).

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excited. The spectra shown in Figure 1b were collected for Rhodamine 6G (R6G) sprayed from methanolic solution ($10\ \mu\text{M}$) with an integration time of only 10 seconds. In the region close to the capillary tip, where the largest aerosol droplets occur, the fluorescence maximum of in-plume R6G exhibited no or only a slight shift relative to that in solution ($\lambda_{\text{max, MeOH}} \approx 555\ \text{nm}$). As the laser beam was translated down the plume, a new component started to appear in the spectrum, with a maximum around 505 nm. At some point (about 10 mm from the spray tip), only the 505 nm fluorescence peak remained visible, while that at 555 nm had disappeared (Figure 1b bottom). No changes in the spectral shape could be observed when the laser beam was translated further down, except for a decrease in signal intensity caused by the plume expansion.

Figure 1c shows a photograph of the ESI plume that was taken when the whole plume area was illuminated with a defocused excitation laser beam. The blue color in the Figure is due to laser light scattered by the droplets constituting the plume. Indeed, the larger the droplet size (r), the stronger the scattering ($\approx r^6$). Figure 1b can thus be interpreted in the sense that the spectrum follows the gradual decrease in droplet size. These data rationalize that the point beyond which no further shift of the peak maximum occurs corresponds to the location where gas-phase R6G ions are formed.

To prove the correlation between the spectral evolution observed and the formation of naked ions, we carried out a reference experiment in which LIF of R6G ions was recorded in high vacuum ($10^{-9}\ \text{mbar}$) inside the ion trap of a Fourier-transform ion cyclotron resonance (FTICR) mass spectrometer. The experimental platform devised for such measurements has recently been described.^[10] Complementary FTICR mass spectrometric analysis confirmed that the fluorescence measurements were conducted specifically on gas-phase R6G ions. A remarkable consistency between the LIF spectrum of R6G ions trapped in the FTICR with that obtained at ambient conditions in the lower part of the ESI plume was found (Figure 1d). This finding provides direct evidence that gas-phase ions are present in high abundance in the lower part of the ESI plume at ambient conditions. By adjusting the position of the excitation laser, liquid droplets can be discriminated against effectively, such that isolated ions can be selectively probed. The size of the blue spike in Figure 1c is a characteristic of how fast desolvation is completed and should be minimized for easier access to gaseous ions. It was found that by decreasing the sample introduction rate (below $1\ \mu\text{L min}^{-1}$) and by increasing the high voltage on the ESI probe more efficient sample vaporization is generally achieved.

Unexpectedly, our data also allow direct insight into the mechanisms of the gas-phase ion production by ESI. A fluorescence profile of the ESI plume was recorded for R6G with $0.5\ \text{mm}$ spatial resolution (Figure 2a; each spectrum presented is normalized to its fluorescence maximum). A two-state spectral characteristic of the transition from parent ESI droplets (red curve) to gas-phase ions (violet curve) is observed as the distance from the capillary tip is increased. Two components were deconvoluted from the experimental data, showing maxima at around 505 nm and 555 nm which

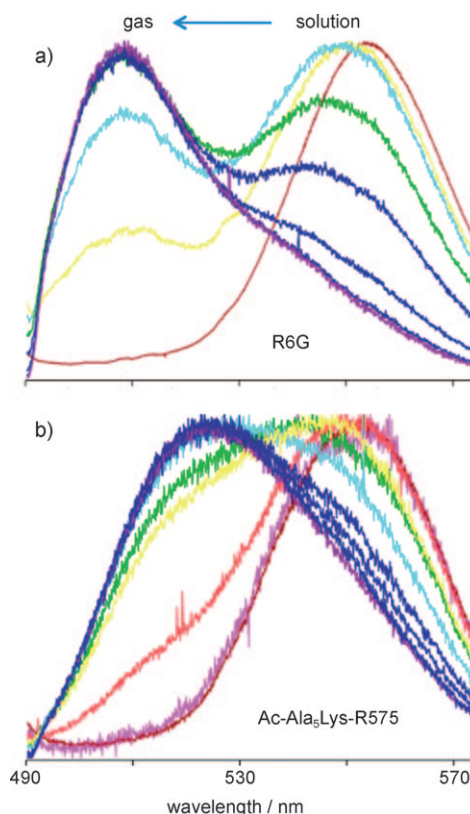


Figure 2. Evolution of the fluorescence spectrum when moving down (from red to violet) along the ESI plume for R6G (top) and Ac-Ala₅-Lys-R575 peptide (bottom).

correspond to the presence of gas-phase ions and solvated species, respectively. Although the positions of the maxima were allowed to shift when the spectra were fitted, a variation of $\leq 3\ \text{nm}$ was obtained for $\lambda_{\text{max, MeOH}}$, while $\lambda_{\text{max, gas}}$ was found to be constant. We predict theoretically (TD-B3LYP/6-31 + G*) that the addition of only one solvent molecule to isolated R6G should shift the absorption maximum by about 2 nm. No evidence for the formation of partially solvated analytes, with a fluorescence maximum at an intermediate wavelength (between fully solvated and gas phase), was found, suggesting that their concentration was very low in the plume. It can therefore be proposed that gas-phase R6G ions are preferentially formed directly from the liquid droplets (ion evaporation model^[11]) rather than by gradual solvent evaporation (charged residue model^[12]).

We also investigated the possibility of selectively accessing gaseous biological molecules using our method. The peptide Ac-Ala₅-Lys was chosen as a model compound; Ac-Ala_n-Lys peptides are the subject of numerous theoretical and experimental studies because of the high proposed stability of their helical structure in the gas phase.^[8,13,14] In the condensed phase, where the internal stabilizing factors are dominated by interactions with solvent, these short peptides adopt randomized conformations.^[15] Ac-Ala₅-Lys was labeled with a fluorescent tag, Rhodamine 575 (R575), at the lysine side chain. In analogy to the R6G experiment, the fluorescence of the peptide was found to shift as the distance from the

capillary tip was increased (Figure 2b). Again, starting from a certain point, no further change in the spectrum could be seen, indicating that formation of gas-phase ions was complete; this was also confirmed by FTICR MS combined with trapped-ion LIF spectroscopy. The mechanism of ion formation was found to be similar to that for R6G as no indication of partially solvated species could be found in the spectra. Interestingly, the gas-phase fluorescence of the R575-tagged Ac-Ala₅-Lys was found to be significantly shifted relative to the gas-phase fluorescence of R575 ions ($\lambda_{\text{max}} \approx 507$ nm). Both the order of magnitude (roughly 650 cm^{-1}) and the direction (towards longer wavelengths) of this shift can be explained by the Stark effect arising from a helix macrodipole.^[16]

The sensitivity achieved when ESI-produced gas-phase species are probed directly at ambient conditions is substantially higher than that of any setup involving trapped ions. This is because the number of ions that can be probed by MS is limited by the trapping capacity of the MS instrumentation, which is generally on the order of a few millions. In contrast, we estimate the total number of gaseous ions instantaneously present in the plume to be on the order of 10^9 – 10^{10} ions. This estimate was obtained by measuring the electrical current on the counter electrode (Figure 1a). These numbers underscore that our method can substantially simplify the spectroscopic characterization of gas-phase biological molecules and can be used for very demanding measurements where the signals are expected to be weak. As an example, we show that despite the low quantum yield of chlorophylls (approximately 0.1) intrinsic LIF spectra can be detected within 10 seconds, using the in-plume strategy (Figure 3). Chlorophylls are unequalled convertors of solar irradiation into “green” electrical energy, and their intrinsic photophysics is therefore of great general interest.^[17] The intrinsic fluorescence is an important characteristic of the conversion efficiency, but is difficult to obtain experimentally. Using LIF on trapped chlorophyll ions in the FTICR MS setup, we failed to obtain any fluorescence signals, probably because of the rapid decomposition of ions in high vacuum upon absorption of light. On the other hand, when one does spectroscopy on ions created at ambient conditions, they are thermalized much more efficiently, and heat-induced dissociation can be

avoided. Also, unlike MS measurements, direct ESI plume spectroscopy deals with ion flows rather than with a trapped-ion population. Therefore, the time over which each ion interacts with the irradiation laser is short (< 1 ms) compared to that in trapped-ion spectroscopy, so that no significant heating occurs. This also allows the use of high laser powers to enhance the signals.

Electrosonic spray ionization (ESSI)^[18] is a variation of ESI in which a supersonic nebulizing gas is employed in order to promote efficient desolvation of the droplets. We profiled an ESSI plume by fluorescence spectroscopy, analogous to the experiments demonstrated in Figure 1. It was found that the desolvation of R6G was complete starting at a distance of roughly 1 cm from the emitter. This observation is very important as it validates the results from several earlier studies that relied on the assumption that ESSI produced isolated gas-phase ions in the ambient, before entry into the mass spectrometer.^[18–21] Owing to the efficient nebulization, ESSI tolerates high rates of sample introduction. We were able to observe gas-phase ions using flows of up to $10 \mu\text{L min}^{-1}$, which allows further enhancement of the achievable gas-phase ion density as compared to ESI. Furthermore, the high linear velocity of an ESSI plume allows one to guide the ion population without any ion optics. Thus, a small fraction of ionic species produced in ESSI can pass a coiled tube without being neutralized, as observed by MS.^[22] We were able to detect gas-phase R6G ions that had passed a 30 cm long coiled tube, although the LIF signal intensity was greatly reduced.

In conclusion, the discovery that high densities of unsolvated nonvolatile ions can be produced inside an ESI plume at ambient conditions substantially enhances the experimental arsenal for probing their intrinsic properties. Isolated biomolecules are accessible for characterization by optical spectroscopy, as well as for further investigation using tandem techniques such as gas-phase reactions,^[20] dissociation,^[22] heating^[19]/cooling,^[23] and soft landing,^[14,24] using low-cost, easy-to-operate equipment.

Experimental Section

A commercial ESI source (Waters, UK) was used. The electric potential was in the range of 3.5–5 kV. The distance between the tip of the emitter and its counter electrode was 2 cm. In ESSI experiments the pressure of the nebulizing gas was 25 bar.

Laser-induced fluorescence was excited by a continuous-wave Ar⁺ laser (Innova 300, the Coherent, USA) with radiation at 488 nm and detected orthogonally by fiber optics coupled to a holographic imaging spectrograph (HoloSpec f/1.8i, Kaiser Optical Instruments Inc, Ann Arbor, USA) with CCD detection (LN/CCD-2500-PB/VISAR, Princeton Instruments, Trenton, USA).

Ac-Ala_n-Lys-R575 was synthesized by Eurogentec (Seraing, Belgium).

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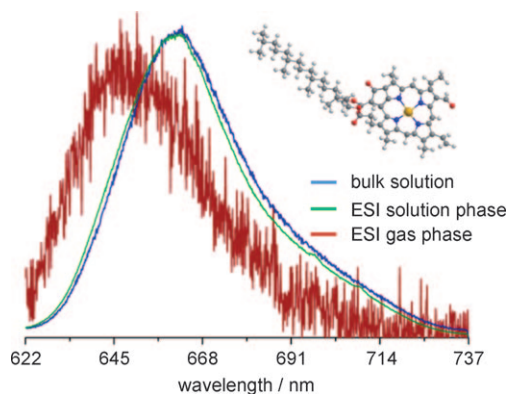


Figure 3. Fluorescence spectra of gas-phase (red) and solution-phase (green) chlorophyll b ions obtained using our setup. The spectrum recorded from bulk solution (blue) is shown for reference.

Keywords: biomolecules · electrospray ionization · gas phase · laser spectroscopy · mass spectrometry

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