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Single droplet separations and surface partition coefficient measurements using laser ablation mass spectrometry

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Abstract

Surface activity of analytes plays a significant role in many chemical and physical phenomena. We present here a mass spectrometric method to characterize surface activity and solute partitioning between bulk liquid and the gas-liquid interface in droplets. The approach employs ablation by an IR laser from the surface of a microliter droplet deposited on a stainless steel post. The ablated material is ionized for mass spectrometric analysis by either droplet charging or by post-ionization in an electrospray plume. Three areas of application have been explored using this method; 1) separations in a single droplet: continuous ablation by a series of many successive laser pulses results in faster depletion of more surface active analytes, effectively comprising a surface activity-based separation. 2) Partition coefficient measurements: droplet volume is held constant during ablation by continually replenishing lost solvent. This leads to analyte-specific ion signal decay curves that may be fitted to a model based on Langmuir adsorption isotherms and simple analytical expressions, yielding quantitative values for the analyte surface partition coefficients. 3) Studies of the relationship between surface partitioning and HPLC phase partitioning: comparisons of surface activities measured by laser desorption with retention times in reversed phase HPLC reveal that the relationship between the two partitioning processes is very sensitive to chemical structure. Poor correlation between the retention time and surface activity is also observed within a subcategory of analytes (peptides). This effect is attributed to multi-modal solute-stationary phase interactions. The laser desorption approach presented here provides direct information on analyte surface activities free from the complications encountered in chromatographic methods due to chemical structure variations.

INTRODUCTION

Liquid droplets play critical roles in many environmental, chemical, and biological processes. Examples include atmospheric chemistry, drug delivery sprays, combustion engines, and a number of analytical methodologies. The importance of droplet-based processes often derives from the dramatic gain in surface-to-volume ratio that occurs when bulk liquid is dispersed into small droplets. The larger surface area affects physical processes such as evaporation, and promotes partitioning of solutes from droplet bulk to droplet surface, enhancing chemical interfacial effects. Our group is particularly interested in the interfacial phenomena occurring in electrospray ionization (ESI).

In ESI, sample solution flows through a sharpened capillary and a high voltage is applied to the capillary tip, resulting in micro- or nanometer size charged droplets. Gaseous analyte

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ions emerge from these charged droplets via mechanisms whose details are still under investigation.[1] These gas-phase ions are then analyzed by a mass spectrometer.

Partitioning of analytes to the surface of the charged droplets has a significant impact on ionization. The more surface-active analytes generally have greater ionization efficiencies and can thus be detected at lower concentration levels.[2-6] It has been proposed that the ion suppression effects and narrow linear dynamic range characteristic of ESI mass spectrometry may be related to the limited surface area available to analyte and matrix components in the droplets.[7] Moreover, the dramatic improvement in sensitivity and matrix tolerance offered by nanospray compared to micro-electrospray is partially due to the increased surface area in nanospray droplets.[8,9]

To study surface effects in ESI-MS both qualitative and quantitative approaches can be useful. For example, in a qualitative study one might order analytes based on their relative surface activities and correlate the results with the relative sensitivities observed in ESI-MS; in a more quantitative approach one might measure the absolute concentrations of analytes on the droplet surface and establish the relationship between surface concentration and absolute sensitivity in ESI-MS. In either case a surface analysis technique would be needed that is capable of analyzing multi-component solutions at the low solute concentrations typical of ESI mass spectrometry.

Analytical techniques for characterizing the air-liquid interface have recently been reviewed.[10] The simplest and most common method of characterizing bulk-interface equilibria is by surface tension measurement. However, this approach does not provide any information on the nature of the species present at the interface, limiting its utility for the analysis of multi-component solutions. Spectroscopic techniques such as second harmonic generation (SHG) and photoelectron spectroscopy (PES) have been developed to address this problem via direct measurement of the solutes at the interface. These techniques, however, are generally not sensitive enough for the study of the micromolar solutions used in ESI. Also, they have other restrictions such as low specificity in the case of SHG and complicated instrumentation and vacuum requirements for PES. Here, we report a laser ablation-based mass spectrometric method for analyzing the air-liquid interface of droplets at atmospheric pressure. The excellent selectivity and sensitivity of mass spectrometry make this technique amenable to the analysis of dilute multi-component solutions. Both qualitative and quantitative analyses of surface partitioning are presented, enabling separations within a single droplet and quantitative determination of surface partition coefficients. The partition coefficients are also compared to retention times in reversed phase HPLC to explore the correlation between the two partitioning processes.

EXPERIMENTAL SECTION

The experimental apparatus is depicted in Figure 1. A micropipette was used to deposit the sample droplet on a 1/16” stainless steel (SS) post located about 5 mm from the extended inlet of an orthogonal time of flight mass spectrometer (Mariner, Applied Biosystems Inc., Foster City, CA). A tunable IR laser (IR Opolette, Opotek Inc., Carlsbad, CA) generated 5-ns pulses of 2940-nm photons which were directed using gold mirrors and focused on the droplet surface by a 1-inch diameter 5-cm focal length CaF_2 lens (Thorlabs Inc., Newton, NJ). The laser pulse energy was about 2 mJ (measured at an 80% setting) at the laser output and the spot size at the sample was an oval shape with major and minor diameters of 620 μm and 430 μm, respectively. The sample droplet on the post was visualized using a TM-200 CCD camera (JAI Pulnix, Sunnyvale, CA) connected to an analog monitor. An orthogonal electrospray plume, emitted from a stainless steel tube (1/16” o.d., 125 μm i.d., Upchurch Scientific, Oak Harbor, WA) recessed 3 mm from a 1-mm thick copper disk with a 1/8” hole

at its center, ionized the ablated material from the droplet surface. The electrospray was sustained by pumping 2.5 μL/min of 1:1 water:methanol + 200-μM formic acid solution through the stainless steel tube using a syringe pump (11 Plus, Harvard Apparatus, Holliston, MA) along with application of 6 kV and 2.5 kV to the SS tube and the copper disk, respectively. The copper disk stabilized the spray and shielded the spray from perturbations that may be caused by the deposition of the ablated material on the electrospray tip.

Test analytes (dodecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, reserpine, CsCl, 5-leu-enkephaline, and a peptide with the sequence Val-Ala-Ile-Thr-Val-Leu-Val-Lys) were purchased from Sigma-Aldrich and used without further purification. Working solutions were prepared from 1-mM stock solutions of the analytes in water, except reserpine. The 1 mM reserpine stock solution was prepared using 20% acetonitrile in water. HPLC grade solvents were purchased from J. T. Baker (Phillipsburg, NJ).

For phase partitioning comparisons, a reversed-phase separation was performed on a 1-mm, 15-cm C18 column (5-μm packing, Phenomenex, Torrance, CA) using a 20-minute gradient at 50 μL/min from 5% to 70% organic solvent provided by an HPLC pump (Ultimate, Dionex, Sunnyvale, CA). The aqueous and organic solvents were 0.1% formic acid and acetonitrile containing 0.1% formic acid, respectively. Analyte detection was effected by coupling the column outlet to an ESI-TOF mass spectrometer (microTOF, Bruker Daltonics Inc., Billerica, MA).

SAFETY CONSIDERATIONS

Special care must be taken in the operation of IR lasers as the laser light is not visible. The use of laser goggles is recommended. In addition, the high voltage connections must be insulated properly to minimize the risk of electrical shock to the operator.

RESULTS AND DISCUSSION

To characterize the surface activity of analytes, particularly peptides, we recently developed a UV-laser desorption technique that is applicable to levitated droplets.[11,12] However, this technique requires large amounts of diethanolamine and UV chromophore as a matrix, severely suppressing the partitioning of analytes to the droplet surface. In the current study, we employed an IR laser tuned to the absorption band of water, eliminating the need for diethanolamine and UV chromophore. Our experimental apparatus is similar to those described by other groups for laser ablation coupled to ESI.[13,14] However, we operate under lower fluences by reducing the laser energy and increasing the laser spot size. Under these conditions, the ablated material is mainly from the surface of the droplet rather than the bulk, leading to direct droplet surface analyses. Material ablation by 2.94-μm IR laser occurs via phase explosion at the droplet surface, releasing microdroplets containing analytes.[13,15] These microdroplets can produce gaseous ions for mass spectrometry either by direct charging of the parent droplet or via coalescence with the ESI plume. Such an approach provides a soft ionization technique where analyte heating is minimal, preserving the molecular structure of the analytes, particularly for fragile molecules such as proteins and peptides. Results are presented below divided into three categories: single droplet separations, quantitative surface partition coefficient measurements, and comparison of surface partitioning with phase partitioning in reversed phase HPLC.

Separations in a single droplet

Figure 2 shows results obtained in the analysis of a mixture of three analytes, dissolved in either 0.1% formic acid in water (Panel A) or 0.1% formic acid in 1:1 water:methanol (Panel B). In this experiment, data acquisition by the mass spectrometer is started at 2 seconds per spectrum. Then a 600-nL droplet containing reserpine, leu-enkephaline and VAITVLVK (1 μM each) is deposited on the SS post. The laser is set to 73% power and 5 Hz repetition rate and manually fired at the start of the 11th spectrum (20 seconds into data acquisition). For 0.1% formic acid solvent (Panel A), reserpine and VAITVLVK appear in the mass spectra immediately after the laser firing because these two analytes partition preferentially to the surface of the aqueous droplet. As the laser ablation continues, the more surface active analytes become depleted from the droplet (about 1.1 minutes into the analysis for VAITVLVK and 1.5 minutes for reserpine). Leu-enkephaline, however, gives rise to a very weak signal in these conditions as it is less surface-active and resides preferentially in the droplet interior. The droplet shrinks during this process due to solvent loss from evaporation and the laser ablation. This causes the less surface active analytes such as leu-enkephaline to become more concentrated as the experiment progresses. To sample such analytes, the laser power is increased to 80% after the surface active analytes are depleted and the droplet height has dropped to ~ 160 μm (from an initial height of ~790 μm). This change leads to increased ablation from the interior of the droplet, resulting in a dramatic enhancement of leu-enkephaline peak in the mass spectra (Figure 2A at about 3.6 minutes). The signal from leu-enkephaline lasts until the droplet is completely ablated by the laser.

The results above show preferential desorption of analytes from the droplet surface, effectively constituting a novel means of separation within a single droplet, based on partitioning of analytes between the droplet interior and surface. This separation technique can also be used to gain insight about buffers more common in ESI. Figure 2B illustrates a similar single droplet separation experiment performed using 50% methanol containing 0.1% formic acid as the droplet solvent. Other experimental conditions for Figure 2B are identical to Figure 2A. The mixed solvent in Figure 2B evaporates faster than the water in Figure 2A, leading to a shorter droplet life time and shorter separation time. Also, methanol evaporates faster than water from the solvent mixture, creating a solvent gradient from high organic to high aqueous content during the analysis, similar to the gradients used in normal phase chromatography. Such a gradient gives rise to two effects. First, the maximum peak intensity for the surface active analytes is shifted to the right in Figure 2B compared to Figure 2A. This effect is attributed to the high organic content of the droplet in the beginning of the experiment, keeping the analytes in the droplet interior and suppressing their surface activity. In other words, it takes some time for the droplet to become enriched enough in water that the hydrophobic analytes prefer the air interface to the droplet interior. Second, the maximum peak intensities for the two surface active analytes are further apart in the gradient separation, revealing that the peptide VAITVLVK is more surface active than reserpine, as it appears earlier in the intensity vs time plots. Note that the timing of leuenkephaline appearance is determined by the manual increase of the laser energy when the sample on the post reached an arbitrarily selected thickness (160 μm). The leu-enkephaline peak thus appears earlier in Figure 2B due to faster evaporation of the sample.

We have also explored another approach to the above experiment in which the ESI source is eliminated, and instead, a voltage of ~2 kV is applied to the SS post. Under these conditions the IR laser pulse produces gaseous analyte ions via ablation of charged droplets which then fission by an ESI-like ionization mechanism.[16,17] However, we have found that the post-ionization using ESI generally provided greater sensitivity, consistent with other studies.[18] Accordingly, the results presented here employed solely the orthogonal ESI sampling approach. Note, however, that the sensitivity and ionization efficiencies do not impact the accuracy of our surface characterization method. This is because the temporal characteristics

of the observed ion signals are dictated by the analyte removal rate via laser ablation from the droplet surface, rather than by gaseous ion generation mechanisms.

The separation of analyte species in a single droplet via laser ablation provides a qualitative picture of analyte partitioning to the droplet surface and may be used to establish the order of surface activity of different analytes. Other mass spectrometric methods have also been developed to probe the liquid-gas interface.[19,20] These methods generally assume that the mass spectrometric ion signals directly reflect analyte surface activities, neglecting other factors that influence gaseous ion generation and detection such as measurement biases caused by differences in mass and/or charge for various analytes. The approach presented here relies on temporal characteristics of mass spectral signals rather than relative signal intensities, obviating the need to know the relationship between surface activity and ion signal intensity.

Quantification of surface partition coefficients

For quantitative analyses, we modified the experimental apparatus to keep the droplet volume constant during the analysis. This was achieved by replacing the SS post with an SS tube having the same o.d ($1/16^{th}$ inch) and a 125 μm i.d., through which solvent was pumped by a syringe pump at 200-400 nL/min, compensating for evaporation and ablation. The droplet was monitored on the CCD camera during the experiment to ensure constant volume conditions.

Figure 3 shows the decay curves obtained for 6 analytes by laser ablation in the constant volume configuration. A 1-μL droplet containing the 6 analytes in water was deposited on the tip of the SS tube. The analyte concentrations (see figure caption) were adjusted to ensure a sufficient ion signal for each, requiring larger concentrations for analytes with lower surface activities. The data acquisition was initiated at 2 seconds per spectrum, followed by actuation of the laser at a 20 Hz repetition rate. Accordingly, each spectrum represents the sum of ions from 40 laser shots. The analyte ion intensities obtained from each spectrum were divided by their corresponding intensities in the first spectrum, resulting in normalized intensities that show a characteristic decay with time (or spectrum number). The signal decay occurs because the analytes are depleted from the droplet surface by the successive laser shots. For a surface active analyte, a larger fraction of the total amount of analyte in the droplet resides on the surface compared to a less surface active solute. Therefore, a larger portion of the surface active analyte is ablated by each laser shot, leading to a faster depletion rate. In other words, the signal decay rates reflect the surface activities of the analytes.

We have developed a model to relate the observed decay rates to the surface partition coefficients. This model is based on three assumptions: 1) analyte partitioning to the droplet surface follows Langmuir's isotherm and adsorption to the SS surface is negligible. 2) The surface partitioning reaches equilibrium between successive laser shots. This assumption is based on our observations from delayed strobe-light imaging of the droplet after each laser shot, indicating droplet oscillations which result in mixing inside the droplet. The internal flow caused by the laser shot was also verified by suspending small particles in the droplet and observing the particle motions inside the droplet (data not shown). 3) The mass spectrometric signal for each analyte is proportional to the amount of analyte ablated from the surface plus the amount of analyte ablated from the interior of the droplet.

Equation 1 describes analyte partitioning to the air-liquid interface based on the Langmuir isotherm:

$$\frac{N_s}{N_s^{\max}}=\frac{N_s\cdot\omega}{S}=\frac{K_L\cdot A_b}{1+K_L\cdot A_b} \tag{1}$$

where N_S is the moles of analyte at the droplet-air interface, N_s^{max} is the maximum number of analyte moles on the surface, ω is the analyte's molar surface (surface area occupied by one mole of analyte), S is the surface area of the air-water interface, K_L is the Langmuir adsorption constant, and A_b is the analyte concentration in the droplet bulk or interior. The concentrations used in our experiments are very low, ensuring that adsorption occurs in the linear range of the Langmuir isotherm. Therefore, the partitioning equilibrium can be simplified to:

$$\frac{N_s}{S}=\frac{K_L}{\omega}\cdot A_b=K\cdot A_b \tag{2}$$

where K is the surface partition coefficient. In other words, Equation 2 defines the surface partition coefficient as the ratio of surface concentration (moles/m^2 in SI units) divided by bulk concentration (moles/m^3 in SI units). For the mass spectrometric signal (p) the equation is:

$$p=\alpha\cdot\left(\frac{S_d}{S}\cdot N_s+V_d\cdot A_b\right) \tag{3}$$

where α is a proportionality constant, and S_d and V_d are the surface area and bulk volume ablated by each laser shot, respectively. The first term in parentheses describes the ion signal contribution from the droplet surface and the second term corresponds to the analyte ion from the droplet bulk. The mass balance equation completes the model:

$$N_s+[A_b]\cdot V_t=C_A\cdot V_t \tag{4}$$

where V_t is the total volume of the droplet (1 μL), and C_A is the average analyte concentration (total analyte moles divided by droplet volume) in the droplet. Combining Equations 2-4 results in Equation 5:

$$p=\alpha\cdot\left(\frac{S_d+\frac{V_d}{K}}{S+\frac{V_t}{K}}\right)\cdot V_t\cdot C_A=\beta\cdot C_A \tag{5}$$

where the mass spectrometric ion signal is related to the analytical concentration with a new proportionality constant β. Considering the amount of the analyte ablated with each laser shot (the term in parentheses in Equation 3) and the constant droplet volume, one can calculate the analytical concentration immediately before each laser shot as a function of the original concentration:

$$C_{A,i}=C_{A,1}\cdot\left(1-\frac{S_d+\frac{V_d}{K}}{S+\frac{V_t}{K}}\right)^{i-1} \tag{6}$$

where the i index indicates the number of the laser shot and $C_{A,1}$ is the original concentration in the droplet deposited on the SS post. The ion signal in the first spectrum (I_1) can be written as the summation of ion signals from M consecutive laser shots:

$$I_1=\sum_{i=1}^{M} p_i=\beta\cdot C_{A,1}\sum_{i=1}^{M}\left(1-\frac{S_d+\frac{V_d}{K}}{S+\frac{V_t}{K}}\right)^{i-1} \tag{7}$$

Similarly, the ion signal in each successive spectrum (I_n) can be described by a summation of ion signals from the corresponding laser shots:

$$I_n=\sum_{i=(n-1)M+1}^{nM} p_i=\beta\cdot C_{A,1}\left(1-\frac{S_d+\frac{V_d}{K}}{S+\frac{V_t}{K}}\right)^{(n-1)M}\sum_{i=1}^{M}\left(1-\frac{S_d+\frac{V_d}{K}}{S+\frac{V_t}{K}}\right)^{i-1}=I_1\left(1-\frac{S_d+\frac{V_d}{K}}{S+\frac{V_t}{K}}\right)^{(n-1)M} \tag{8}$$

Equation 8 indicates a power-law signal decay for each analyte. For the data in Figure 3, 40 laser shots are averaged for each spectrum (M = 40) and the intensities are normalized. Therefore, Equation 8 can be simplified as follows:

$$\frac{I_n}{I_1}=(1-B)^{40(n-1)} \tag{9}$$

$$B=\frac{S_d+\frac{V_d}{K}}{S+\frac{V_t}{K}} \tag{10}$$

where B is a constant that depends on analyte, droplet size, and laser ablation conditions, describing the characteristic decay curves. Note, however, that the measured ion intensity in the first spectrum (I_1) is subject to variation within the measurement precision, complicating the mathematical description of the normalized intensities. To account for this effect, the normalization to the first spectrum can be treated as normalization to an unknown value. Accordingly, the normalized ion intensity in the nth spectrum (R_n) can be described by Equation 11:

$$R_n=R_1\cdot(1-B)^{40(n-1)} \tag{11}$$

where R_1 is the true normalized intensity in the first spectrum. For a precise measurement, the measured I_1 is closer to its true value and R_1 approaches unity.

Equation 11 is fitted to the experimental data for each analyte using R_1 and B as fit parameters in the nonlinear curve fitting tool of a commercial data analysis package (Origin 8, OriginLab, Northampton, MA). The solid lines in Figure 3 represent the fitted Equation and the corresponding fit parameters are summarized in Table 1. As reflected by the R_1 values, our measurements are more precise for analytes with higher surface activity. This is also evident from the levels of data scattering around the fitted curves in Figure 3.

The next step in our analysis is calculating K values using the B values in Table 1 and Equation 10. For this purpose, V_t, S, S_d, and V_d must be known. In our experiments V_t = 1 μL, and the air-water interface area can be calculated considering that the 1-μL droplet on

the 1/16" SS post forms an approximate hemisphere (S = 3.96 mm^2). For the two remaining parameters, two of the analytes are used as standards. Cesium ion has very low surface activity (well solvated) and the amount of cesium ion on the droplet surface is negligible compared to the amount of the analyte in the droplet interior. This means K_{Cs} is very small, implying that the decay in Cs^+ signal is mainly due to ablation of droplet bulk. Mathematically, this translates to $B_{Cs} \approx Vd / Vt$, leading to a value of 23.6 pL for V_d. Hexadecyltrimethylammonium (HDTMA) is the most surface active analyte in this study and was selected as the second standard. Also, the adsorption of HDTMA bromide to air-water interface has been well characterized in the literature using surface tension measurements. Stubenrauch et. al. recently reported K_L and ω values for several surfactants via fitting their molecular compressibility model to surface tension data.[21] We have used the values for HDTMA bromide in their study to calculate K_{HDTMA} (Equation 2). Applying this value to Equation 10 along with the parameters determined above leads to a value of 0.63 mm^2 for S_d. Accordingly, all the parameters in Equation 10 are calibrated using two standards and can be used to calculate the surface partition coefficients for the rest of the analytes (Table 1).

The consistency of our results with other studies may be assessed by comparing the partition coefficient of dodecyltrimethylammonium (DDTMA) in Table 1 (7.42×10^{-7} m) with that reported in Reference [21] (5.50×10^{-7} m). Our results yield a value 35% greater than the previously reported value, which was obtained from a molecular compressibility model applied to data from surface tension measurements. However, one must note that the values obtained from surface tension measurements depend upon the nature of the model used to interpret the data. For example, Stubenrauch et. al. reported a 38% lower value for the DDTMA partition coefficient when applying the Frumkin model to the same surface tension data as for the compressibility model.[21] Considering this variation in reported results, our results appear to provide acceptable values consistent with the surface tension measurements found in the literature. This consistency with surface tension data also attests to the validity of our model's assumptions.

Correlation of partitioning to droplet surface and phase partitioning in HPLC

Retention times in reversed phase HPLC are commonly used to characterize the surface activity of analytes because of the experimental difficulties in surface partitioning characterization.[4,22] However, the interaction between solutes and the stationary phase in HPLC is often complicated by other forces such as the electrostatic interaction of charged analytes with polar or charged groups on the stationary phase. Therefore, using chromatographic retention times may lead to erroneous conclusions about surface activity of analytes. In contrast, the laser ablation mass spectrometric method presented here samples the analytes directly from the surface of a water droplet, and is accordingly not subject to such chromatographic complications. This effect is shown in Figure 4 where B factors and corresponding K values of several analytes are plotted against the corrected retention times on a C18 column. The ordinate represents partitioning of a solute to a droplet surface and the abscissa is a measure of partitioning to a C18 solid phase. As depicted in Figure 4, there is no correlation between the results obtained from the two methods for the analytes used in this study. The case of two peptides is particularly interesting. VAITVLVK elutes earlier than leu-enkephaline, however, it shows a larger surface activity based on laser desorption data. The higher surface activity is consistent with our measurements using ESI, where VAITVLVK shows larger sensitivity (data not shown). This reflects the complexity of interactions in chromatographic separations even within a subcategory of chemical structures, underscoring the importance of the measurement method presented here for the evaluation of surface activities.

CONCLUSIONS

It is shown here that IR laser ablation from a droplet surface provides important information about the surface activity of analytes. The laser ablation may be performed either without or with solvent addition for qualitative and quantitative analyses, respectively. In the former case, the droplet shrinks during analysis, preferentially depleting the more surface active analytes while concentrating the more polar analytes in the droplet interior. In cases where the droplet solvent is a organic:aqueous mixture, the more volatile component (typically the organic component, e.g. methanol) is depleted more rapidly, causing the droplet solvent composition to become more polar with time and thereby changing the surface partition coefficients. This process is a single droplet analog to the solvent gradients employed in liquid chromatography, and provides a simple qualitative means of assessing analyte surface activities. However, the changes in droplet volume and solvent composition make quantitative analyses problematic. To eliminate these variables and enable quantitative analyses in the case of single solvent systems, solvent may be continuously added to the droplet to maintain a constant volume. In this configuration, the laser desorption process causes analyte-specific depletion rates with the more surface active analytes depleted at a higher rate. We have developed a quantitative model for this process that yields values for the analyte surface partition coefficients. It is shown that the values measured in this manner do not correlate well with the retention times of analytes on a C18 column across a wide range of chemical structures. This is presumably due to the multi-modal nature of solute-stationary phase interactions, which complicate measurements of surface activity based on retention times, highlighting the importance and utility of more direct measurements such as those described here. This work opens new avenues for the study of ionization efficiency in ESI, where surface activity is an important parameter in determining detection sensitivity. Historically, the relationship between ionization efficiency and surface activity has been studied by correlating chromatographic retention times with analytical signal. However, this approach, as indicated above, is only applicable to analytes with very similar chemical structures, where the retention time is proportional to surface activity. Our new method eliminates this restriction, providing quantitative correlations between surface activity and ESI response across a wide range of analytes. This approach can reveal the effect of other molecular properties (such as charge) on ESI response, greatly enhancing our understanding of the ESI ionization process.

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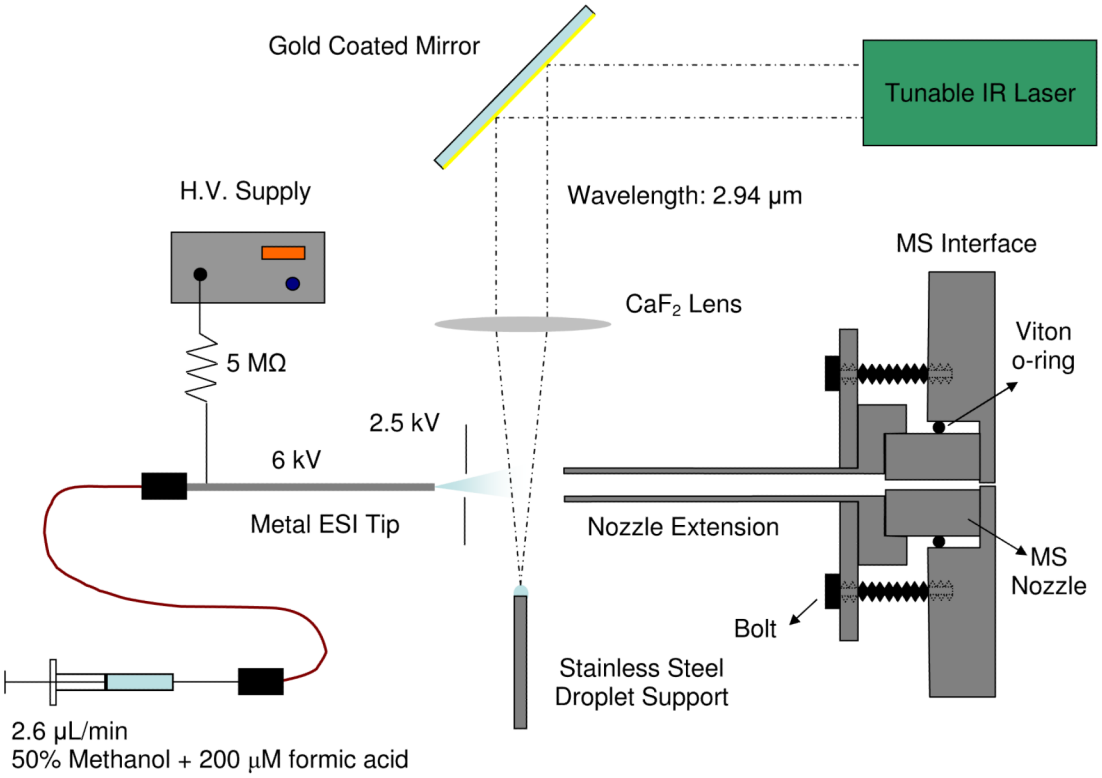

Figure 1.
Experimental apparatus for IR laser ablation electrospray ionization of droplets.

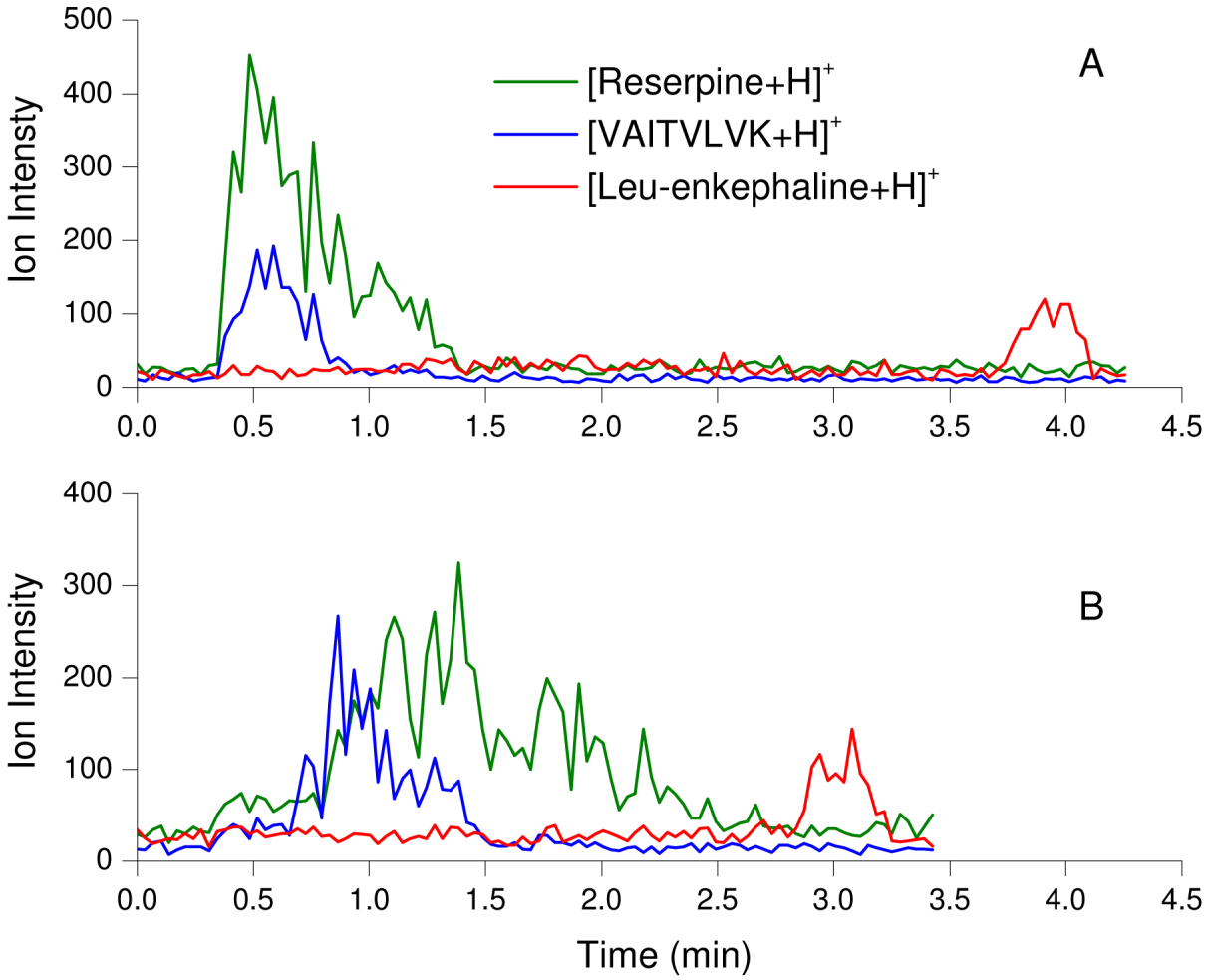

Figure 2.

Ion intensities as a function of time illustrating surface partitioning-based separations in a single 600-nL droplet using laser ablation from the droplet surface: A) 0.1% formic acid solvent, B) 50% methanol+0.1% formic acid solvent. The laser is operated at 5 Hz and mass spectral data acquisition is performed with a 2 second integration time. The droplets contain an equimolar mixture of three analytes (1 μM each).

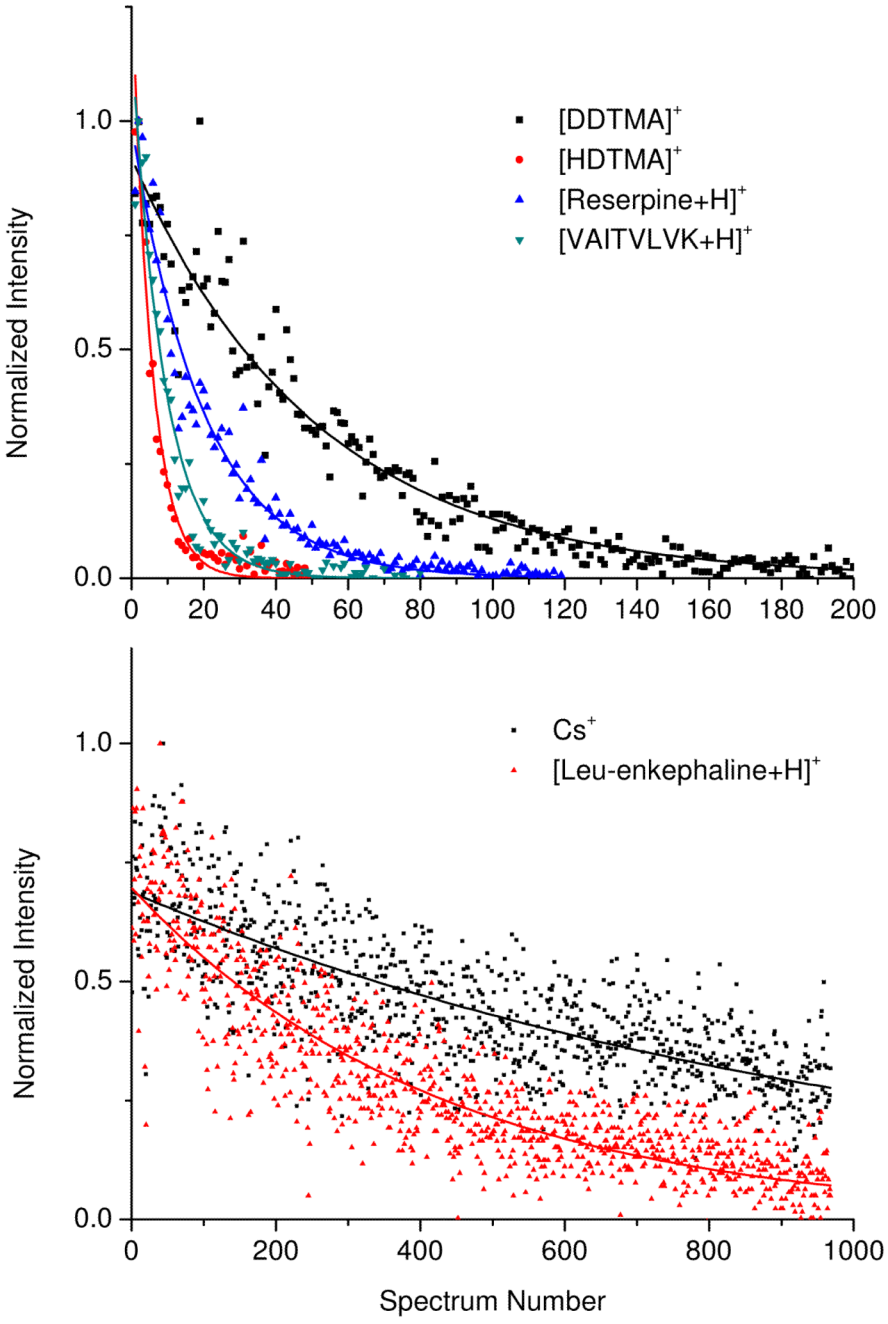

Figure 3.

Surface partitioning-dependent decay curves generated by laser ablation from a droplet surface maintained at constant droplet volume. The sample is a 1 μL water droplet containing 250 nM hexadecyltrimethylammonium (HDTMA), 250 nM dodecyltrimethylammonium (DDTMA), 1 μM reserpine, 1 μM VAITVLVK, 10 μM leu-enkephaline, and 100 μM cesium. The laser is operated at 20 Hz and data acquisition is performed at 2 seconds per spectrum. The data in the two panels were acquired simultaneously; however, cesium and leu-enkephaline take much longer to deplete and are thus plotted separately for clarity of the Figure (note the different scales for the abscissas in top and bottom panels). The solid lines are fits of our decay model (Equation 11) to the data.

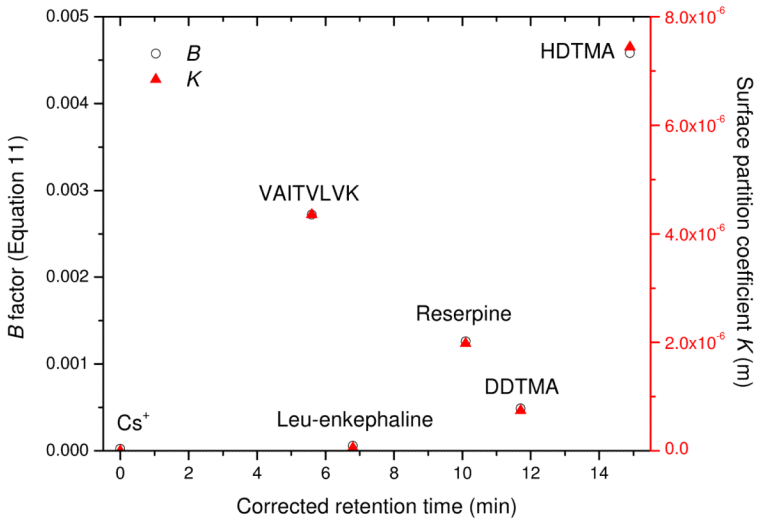

Figure 4.
Correlation of droplet surface partitioning (ordinates) with phase partitioning in reversed phase HPLC (abscissa).

Table 1

Fit parameters for Equation 11 and resulting partition coefficients.

Analyte	R_1	B	K(m)
Hexadecyltrimethylammonium	1.10	4.58×10^{-3}	7.44×10^{-6} *
VAITVLVK	1.05	2.72×10^{-3}	4.35×10^{-6}
Reserpine	0.95	1.26×10^{-3}	1.98×10^{-6}
Dodecyltrimethylammonium	0.90	4.90×10^{-4}	7.42×10^{-7}
Leu-enkephaline	0.70	5.89×10^{-5}	5.61×10^{-8}
Cesium	0.69	2.36×10^{-5}	~ 0

* this value is taken from reference 21