

*Chapter 1*

## INTRODUCTION

(Reproduced in part with permission from Thomas, D. A.; Wang, L.; Goh, B.; Kim, E. S.; Beauchamp, J.L. *Anal. Chem.* **2015**, 87, 3336-3344. <http://dx.doi.org/10.1021/ac504494t>  
Copyright 2015 American Chemical Society)

**1.1 Background**

The liquid/vapor interface is one of the most ubiquitous chemical environments in nature, present on the surface of oceans and lakes,<sup>1</sup> on cloud and fog waters,<sup>2-4</sup> on aqueous deliquescent particles,<sup>5,6</sup> and in the human respiratory system.<sup>7,8</sup> As a result of its singular attributes including an immense change in density on molecular length scales,<sup>9</sup> the existence of a localized electrical potential,<sup>10-13</sup> and the presence of free surface hydroxyl groups,<sup>14</sup> this intriguing environment often serves as a medium for unique processes unobserved in bulk phases.<sup>1,7,15-17</sup> In the earth's atmosphere, processes such as adsorption of gas-phase molecules,<sup>3,9,18-22</sup> surface partitioning of amphiphilic or highly polarizable analytes,<sup>9,11,23,24</sup> and heterogeneous reactions between dissolved organics and gas-phase oxidants<sup>17,25-28</sup> can strongly influence the chemical evolution and properties of aqueous aerosol. In spite of the pervasiveness of the liquid/vapor interface, numerous attributes of this system remain poorly understood, with even the properties and structure of the neat water/air interface still the subject of much debate.<sup>29</sup>

The study of chemical or structural changes occurring at interfaces has been difficult, as the selective detection of molecules in the interfacial environment and their differentiation

from the underlying bulk phase is not straightforward. Specialized experimental methodologies are required to specifically study the chemistry occurring at the liquid/vapor interface, and among the most popular of these methods are the nonlinear optical spectroscopy techniques of vibrational sum frequency generation (VSFG) and second harmonic generation (SHG) spectroscopy, in which transitions are forbidden in media with inversion symmetry (i.e., bulk phases), and the spectrum is therefore dominated by interface-specific surface resonances.<sup>30,31</sup> These techniques, in combination with theoretical simulations,<sup>11,12,32-34</sup> have been employed to study the structure of neat water at the interface,<sup>35</sup> the surface enhancement of hydronium and hydroxide ions,<sup>36,37</sup> the surface properties of inorganic ions,<sup>38-40</sup> and the orientation of molecules in the interfacial region.<sup>41-43</sup> Other interface-specific spectroscopic methods, including infrared-reflection absorption spectroscopy,<sup>44-46</sup> glancing-angle spectroscopy,<sup>47-49</sup> and Brewster angle microscopy<sup>50-54</sup> have also provided insight into the orientation and arrangement of molecules at the water surface. In addition, the properties of liquid/vapor interfaces have been studied recently by photoelectron spectroscopy of liquid jets,<sup>55-58</sup> neutron reflection from a liquid surface,<sup>59</sup> and grazing incidence X-ray diffraction of surface monolayers.<sup>53,60-62</sup> To study the dynamics and kinetics of exchange at the interface between gas- and liquid-phase species, droplet train flow reactor (DTFR) and molecular beam scattering experiments have been employed.<sup>20,63,64</sup>

Though the above methodologies yield extensive information on the structure and properties of the liquid/vapor interface, their application to the study of time-dependent, multicomponent chemical systems is challenging. Nevertheless, VSFG spectroscopy in particular has been successfully applied to the study of several chemical systems, including

the uptake and reaction of methanol on sulfuric acid solutions,<sup>19</sup> the oxidation of oleic acid by ozone,<sup>27</sup> and the uptake of SO<sub>2</sub> from the gas phase and subsequent reaction in aqueous solution.<sup>65-67</sup> In addition, the heterogeneous chemistry of model atmospheric aerosols has been studied by online chemical ionization mass spectrometry of organic particles,<sup>68-70</sup> VSFG of model sea spray aerosol,<sup>71</sup> and detection of products in large aerosol chambers.<sup>11,15</sup>

Several methods utilizing atmospheric pressure ionization mass spectrometry have also been developed recently to study the dynamics of chemical reactions at the water/air interface. Colussi, Hoffmann, and co-workers developed a technique based on sonic spray ionization (SSI)<sup>72</sup> for the analysis of interfacial reactions. Droplets in an aqueous microjet produced by SSI are exposed to gas-phase reactants and subsequently sampled by transfer through the atmospheric pressure interface of a mass spectrometer. This method has been used to examine several interfacial processes, including the reaction of halogen anions and organic species with ozone and the availability of hydronium ions at the interface.<sup>73-78</sup> In addition, surface-selective sampling of analytes has been achieved by laser ablation of droplets on a rod or suspended by acoustic levitation.<sup>79-82</sup>

The technique of field-induced droplet ionization mass spectrometry (FIDI-MS), developed by Grimm and co-workers, has been utilized for the time-resolved examination of interfacial reactions between a hanging droplet and gas-phase reagents.<sup>83</sup> In this method, the application of a strong electric field induces prolate elongation of a droplet, generating symmetric negatively and positively-charged Taylor cone-jets of highly charged progeny droplets, which are subsequently sampled by a mass spectrometer.<sup>84,85</sup> FIDI-MS has been applied to the investigation of oxidative damage in the lungs caused by the exposure of

various components of the lung surfactant system to ozone, highlighting the ability of this technique to analyze complex processes such as ozonolysis that are common at liquid/vapor interfaces.<sup>7,86,87</sup>

The studies presented in this thesis continue the investigations initiated by Grimm and co-workers into heterogeneous reactions occurring at the surface of liquid droplets. Chapter 2 details experiments examining base-catalyzed reactions occurring at the liquid/vapor interface upon uptake of ammonia from the gas phase. In addition, the FIDI apparatus has been redesigned and coupled to a new mass spectrometer to increase sensitivity and enable the exploration of more complex chemical systems. The design and fabrication of an enclosure for the source allows for greater control of ambient conditions during FIDI experiments, and this new apparatus is utilized in Chapter 3 to study the oxidation of glycolaldehyde initiated by photolysis of iron (III) oxalate complexes. Chapter 4 details the development of alternative methods for mass spectrometric sampling of the liquid/vapor interface, with special focus on the ejection of nanoliter droplets from the surface by focused acoustic waves.

The studies detailed in Chapters 5 and 6 do not address the chemistry of the liquid/vapor interface; instead, these chapters examine the application of gas-phase free radical chemistry to the sequencing of biomolecules. Specifically, Chapter 5 explores the mechanism of free-radical-initiated dissociation observed at serine and threonine residues in peptides, and Chapter 6 details the combination of a free-radical-initiated peptide sequencing (FRIPS) and ion mobility-mass spectrometry (IM-MS) for the separation of isomeric peptides.

## 1.2 Contents of Thesis

### 1.2.1 Base-Catalyzed Chemistry at the Liquid/Vapor Interface

Recent studies on the rapid unfolding and refolding of proteins in electrospray droplets exposed to gaseous acids and bases<sup>88,89</sup> prompted us to examine if similar conditions could initiate chemical changes at the liquid/vapor interface of a hanging droplet. As the uptake of gaseous acids and bases by aqueous particles is an important atmospheric process, such reactions also yield insight into the processing of tropospheric aerosol. Ammonia ( $\text{NH}_3$ ) is the primary volatile base found in the atmosphere and thus plays an especially important role in atmospheric chemistry. Chapter 2 explores the dissociation of a dissolved base-cleavable surfactant upon exposure of pendant droplets to gas-phase  $\text{NH}_3$ . Importantly, the concentration of ammonia utilized in these experiments is insufficient to modulate the bulk droplet pH, yet significant surfactant dissociation is still observed, suggesting that unique conditions at the liquid/vapor interface increase the rate of this reaction. A simple model of analyte uptake into a droplet coupled with reaction and diffusion suggests that an increase in pH near the surface of the droplet occurs upon absorption of  $\text{NH}_3$  from the gas phase.

### 1.2.2 Photochemistry of Iron (III) Oxalate: Oxidation of Organics

As an abundant inorganic component of atmospheric aerosol, iron is known to undergo many key redox processes that exert a strong influence on the composition and properties of tropospheric particles. For example, iron catalyzes the oxidation of sulfur dioxide to sulfate in mineral dust aerosols, resulting in the removal of sulfate from the atmosphere by aerosol deposition and altering the estimated radiative forcing of tropospheric sulfate

aerosol.<sup>90</sup> Dark Fenton chemistry and photo-Fenton chemistry, both of which produce hydroxyl radicals by iron redox processes in solution, have also been suggested as important processes in tropospheric aerosol.<sup>91</sup> In addition, the complexation of iron (III) with oxalic acid in aqueous solution results in the reduction of iron with high quantum yield and the generation of hydroxyl radicals.<sup>92</sup> Recent field experiments found an inverse correlation between the concentration of iron and oxalic acid in cloud waters in the northwest Pacific Ocean, suggesting this chemistry may also be operative in tropospheric systems.<sup>93</sup> In Chapter 3, we study the oxidation of glycolaldehyde, a model semi-volatile organic compound abundant in the troposphere, initiated by photodissociation of iron (III) oxalate complexes. We monitor the consumption of oxalic acid and identify the oxidation products generated in real time by FIDI-MS. The utilization of an enclosed FIDI source provides a means to control ambient conditions, and experiments conducted in a deoxygenated environment support the proposed mechanisms of reaction.

### **1.2.3 Alternative Methods for MS Sampling of Liquid Surfaces**

Although FIDI-MS has proven an extremely effective method for the study of heterogeneous reactions occurring on a time scale of tens of seconds to minutes, the inability to sample from planar surfaces using this technique prompted the study of alternative sampling methods. The coupling of mass spectrometric sampling with surface pressure measurements in a Langmuir trough or similar device would provide real-time information on both the physical properties and chemical composition of a liquid surface over the course of a reaction. Toward this goal, Chapter 4 introduces the sampling techniques of bursting bubble ionization (BBI) and interfacial sampling with an acoustic

transducer (ISAT), in which droplets (0.5-10 nL volume) are ejected from the surface of bulk solution. In BBI, these droplets are generated at the tip of a liquid jet that fills the cavity left by a bursting bubble at a liquid surface. Utilizing ISAT, droplets are ejected on demand by piezoelectrically generated acoustic waves focused at the liquid/vapor interface. Experiments show that droplets generated by either method are near-neutral in net charge, suggesting that the production of gas-phase ions occurs by the breakup and charging of droplets during transfer through the atmospheric pressure interface of the mass spectrometer, similar to the process proposed for sonic spray or thermospray ionization. As a proof of concept for the study of heterogeneous reactions by ISAT-MS, we present results from experiments on the oxidation of aqueous oleic acid by ozone impinging upon the liquid surface from the gas phase.

#### **1.2.4 Mechanism of Radical-Initiated Dissociation at Serine and Threonine**

Free-radical-initiated peptide sequencing (FRIPS) is an alternative method for the dissociation of gas-phase peptide ions within a mass spectrometer that takes advantage of the unique reactions of free radicals to gain peptide sequence information. In its most recent incarnation, a free radical precursor is coupled to a peptide or protein, and gas-phase collisional activation results in the selective formation of an acetyl radical at the N-terminus by homolytic bond cleavage. This radical then initiates dissociation by abstraction of a hydrogen atom, typically from either  $C_\alpha$  or  $C_\beta$  of the peptide side-chain. Recent experiments by Sohn and co-workers demonstrate nearly 100% sequence coverage utilizing this method in peptides that are challenging to sequence by standard collisional activation methods.<sup>94</sup> Anomalous dissociation patterns are observed at serine and threonine

residues within a peptide chain during FRIPS experiments. Chapter 5 details extensive experimental and theoretical studies of the mechanism of dissociation in the presence of these amino acids. It is found that hydrogen bonding between the side-chain hydroxyl group and backbone amide carbonyl results in distinct low-energy dissociation pathways that give rise to the anomalous product ions observed in FRIPS spectra.

### **1.2.5 Separation of Isomeric Peptides by FRIPS and IM-MS**

The implementation of ion mobility spectrometry in combination with mass spectrometry (IM-MS) provides an additional degree of separation that greatly improves the analysis of complex samples. In proteomics experiments, the isolation of peptides with strong sequence homology, which are challenging to separate by liquid chromatography alone, may yield significantly more insight into the expression levels and role of cellular proteins. Most IM-MS systems integrate the ion mobility device directly into the mass spectrometer or require extensively customized instrumentation. A new ion mobility device, the radial opposed migration ion and aerosol classifier (ROMIAC), developed by Mui and co-workers, is relatively simple to fabricate and easily integrated with the atmospheric pressure interface of most commercial mass spectrometers.<sup>95</sup> Chapter 6 details some of the initial studies performed using the ROMIAC. Specifically, the ROMIAC is used to separate isomer peptides as a demonstration of its utility in the analysis of complex peptide samples. In addition, the tagging of peptides with the FRIPS reagent is found to enhance peptide separation during IM-MS experiments.