

RESEARCH ARTICLE

Multistage Reactive Transmission-Mode Desorption Electrospray Ionization Mass Spectrometry

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science such as catalyst development. It is often difficult to probe fast reactions at ambient conditions with high temporal resolution. In addition, systems involving reagents that cross-react require analytical methods that can minimize interaction time and specify their order of introduction into the reacting system. Here, we explore the utility of transmission mode desorption electrospray ionization (TM-DESI) for reaction monitoring by directing a microdroplet spray towards a series of meshes with micrometer-sized openings coated with reagents, an approach we call multistage reactive TM-DESI (TMⁿ-DESI, where *n* refers to the number of meshes; n=2 in this report). Various stages of the reaction are initiated at each mesh surface,

Abstract. Elucidating reaction mechanisms is important for advancing many areas of

generating intermediates and products in microdroplet reaction vessels traveling towards the mass spectrometer. Using this method, we investigated the reactivity of iron porphyrin catalytic hydroxylation of propranolol and other substrates. Our experimental results indicate that TMⁿ-DESI provides the ability to spatially separate reagents and control their order of introduction into the reacting system, thereby minimizing unwanted reactions that lead to catalyst deactivation and degradation products. In addition, comparison with DESI-MS analyses (the Zare and Latour laboratories published results suggesting accessible reaction times <1 ms) of the reduction of dichlorophenolindophenol by *L*-ascorbic acid suggest that TM¹-DESI can access reaction times less than 1 ms. Multiple meshes allow sequential stages of desorption/ionization per MS scan, increasing the number of analytes and reactions that can be characterized in a single experiment.

Keywords: Ambient mass spectrometry, Transmission-mode desorption electrospray ionization, Reaction monitoring, Mechanisms, Time scale, Catalysis, Iron porphyrin

Received: 25 February 2015/Revised: 16 April 2015/Accepted: 18 April 2015/Published Online: 20 June 2015

Introduction

C haracterizing reaction mechanisms is important for developing new catalysts, drugs, and materials that address scientific and socioeconomic problems[1]. However, it is often difficult to obtain detailed molecular mechanistic information under normal operating conditions because intermediates have short lifetimes (typically less than ~1 s), exist in a complex and dynamic matrix involving multiple reaction pathways, and have low concentrations [2–5]. These difficulties are significantly more pronounced for homogenous multi-catalytic systems [6], which show great promise for rapidly achieving one-

Electronic supplementary material The online version of this article (doi:10.1007/s13361-015-1171-5) contains supplementary material, which is available to authorized users.

pot complex transformations that improve efficiency, selectivity, and enantiomeric purity. Advancing these areas of research demand analytical technologies that can capture fleeting intermediates in real-time at ambient conditions, as well as provide the ability to separate reagents and specify their order of introduction to minimize unwanted side reactions between incompatible reagents during analysis and to facilitate step-wise elucidation of reaction mechanisms.

Electrospray ionization mass spectrometry (ESI-MS) methods are one of the primary approaches for obtaining realtime information about solution-phase molecular species formed in the course of a reaction with high sensitivity, speed, and selectivity [7]. The recent introduction of ambient mass spectrometric techniques[8–15] such as desorption electrospray ionization (DESI)[16] developed by Cooks and co-workers has revolutionized analytical chemistry in the past decade, allowing chemical analyses with minimal sample preparation. A recent advance developed by Zare and co-workers

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(a)

involves using DESI to capture short-lived solution-phase reaction intermediates (<1 ms) [17–23] at ambient conditions, while minimizing sample preparation times, carry over effects, and experimental complexity compared with ESI configurations. This elegant discovery has opened the possibility for the development of new types of ambient ionization sources and applications for characterizing fast solution-phase processes [4, 24–32].

Transmission-mode DESI (TM-DESI)[33–38], developed by Brodbelt and co-workers, is an ambient MS method that involves directing an electrostatically charged solvent spray at a mesh having micrometre-sized open areas on which analytes of interest are deposited. TM-DESI requires small sample volumes and minimal source optimization prior to analysis, making it easily amenable to high-throughput analyses[33, 36, 39, 40]. TM-DESI has been previously used for direct analysis of samples containing analytes such as peptides and small organic molecules [33]. In addition, the mesh can be functionalized for selective extraction of analytes from complex matrices followed by TM-DESI-MS [35, 40]. Despite its simplicity, the utility of TM-DESI for probing chemical reactivity has not yet been explored.

Herein, we describe a TM-DESI-based ionization source that employs two meshes in series (M1 and M2) (Figure 1), referred to hereafter as multistage reactive transmission-mode DESI (TM^n -DESI, where *n* represents the number of desorption stages; conventional TM-DESI configuration has n=1) for characterization of reaction mechanisms. Using TM²-DESI-MS, we studied iron (Fe) porphyrin-catalyzed hydroxylations such as Fe tetra(pentafluorophenyl)-porphyrin (Fe-TPFPP, 1) hydroxylation of substrate propranolol 2 (proposed mechanism shown in Scheme 1 [41]), previously analyzed by ESI Fourier transform ion cyclotron resonance MS[42, 43]. Experimental results show that TM²-DESI provides the ability to spatially separate reagents and to specify their order of introduction into microdroplet reaction vessels, reducing off-path processes such as oxidant-mediated hydroxylation of 2 (Scheme 1). In addition, comparison of TM¹-DESI-MS and DESI-MS analyses of the chemical reduction of dichlorophenolindophenol (DCIP) by L-ascorbic acid (L-AA; Figure 3; reaction previously analyzed by liquid DESI-MS[44]) showed that TM¹-DESI can access reaction times less than 1 ms. These capabilities, coupled with the high-throughput features of TM-DESI, demonstrate a unique ambient ionization approach for chemical analyses at ambient conditions. Multiple meshes increase the number of analytes and reaction steps that can be characterized per MS scan by a factor of n, which has great potential for reducing analysis time and increasing high-throughput analyses at ambient conditions.

Experimental

Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification, except for the



Mesh 1 (M1)

mCPBA

Mesh 2 (M2)

Desorbed microdroplets

MS. (b) Three-dimensional representation of TM -DESI-MS. (b) Three-dimensional rendition showing the meshes in front of the MS inlet (for clarity, the micromanipulators that attach the sprayer holder to the stage and the alignment bar are not shown; their locations are indicated with dashed lines). Inset shows a close-up 3D view of the rotatable posts, mesh holder, and alignment bar. These images are not drawn to scale

Fe porphyrin catalysts (Fe tetra(pentafluorophenyl)porphyrin, Fe-TPFP, 1; Fe tetraphenylporphyrin, Fe-TPP; Fe tetramesitylporphyrin, Fe-TMP; Fe tetra(2,6dichlorophenyl)porphyrin TDCPP, Fe-TDCPP; Fe tetra(2,6difluorophenyl)porphyrin, Fe-TDFPP), and propranolol 2, which were purchased from Frontier Scientific, Inc. (Logan, UT, USA). The polyether ether ketone (PEEK) meshes (71 μ m strand diameter, 56% open area) were purchased from Small Parts, Inc. (Miramar, FL, USA). PEEK was chosen as the mesh material because of its high chemical resistance to dichloromethane (CH₂Cl₂) compared with other polymers such as polypropylene.

Design of the Multistage Reactive Transmission Mode Desorption Electrospray Ionization Source

The microdroplet sprayer of the TM^{*n*}-DESI source has similar specifications to the home-built DESI source described by Cooks and co-workers[45]. Briefly, the sprayer consists of a Swagelok (Swagelok Company, Fremont, CA, USA) 1/16'' stainless steel (SS) tee through which a fused silica (FS1) capillary (75 µm internal diameter (i.d.); 190 µm outer diameter (o.d.); Polymicro Technologies, Phoenix, AZ, USA) delivers



Scheme 1. (a) Catalytic cycle for Fe-TPFPP oxidation of propranolol. Species 1a and 1c (red) could not be unambiguously identified because they are isobaric with degradation products having oxygen insertion into the porphyrin ring. (b) Degradation pathway of Fe-TPP from reference [55]

liquid from a Harvard Apparatus Standard Pump 22 (Holliston, MA, USA) syringe pump. FS1 passes through a 1/16" fluorinated ethylene propylene tubing (i.d.: 200 µm; length: 5 cm; IDEX Health and Science, Oak Harbor, WA, USA) that holds FS1 in place with a stainless steel (SS) nut and ferrule. The other end of FS1 passes through a second FS capillary (FS2; length= \sim 1.5–2 cm; i.d. = 250 μ m; o.d. 360 μ m), which is held in place with a graphite ferrule and SS nut. FS1 and FS2 protrude from the end of the SS nut by ~0.7 and 0.5 cm, respectively. The FS1-to-MS inlet distance ranges from 5 mm to 13 mm (Figure 1a). Sheath gas (nitrogen; N₂) enters the sprayer at the 90° opening of the tee (line pressure= $\sim 150-200$ PSI) and exits FS2, generating the microdroplet reagent spray. Measurements of $[2+H]^+$ (*m/z* 260.164) intensity as a function of N_2 line pressure show that a threshold value of ~150 PSI is necessary for efficient desorption/ionization from M2 in TM²-DESI (Supplementary Figure S1a). The sprayer is connected to a 1/16" Swagelok bulkhead union that is mounted in an aluminium bar (sprayer holder; Figure 1b). The sprayer holder is

connected to *x*, *y*, and *z* micromanipulators (location shown by dotted lines in Figure 1b) mounted on a modified PicoView 550 (New Objective, Inc., Woburn, MA, USA) stage. The stage mounts on the frame of an LTQ-Orbitrap XL mass spectrometer[46–51] via a pair of latches (Figure 1b).

Each mesh is held in place by a pair of rotatable posts (6.5 cm in height) that are secured to separate mesh holders via set screws (Figure 1b). The rotatable posts provide control of mesh position and tension, which are critical parameters for obtaining reproducible measurements; a loose mesh has erratic motion in the microdroplet plume, which causes large variations in signal intensity at a given concentration of analyte. The rotatable posts are composed of two segments that can be clamped together by a pair of screws (Figure 1b inset) to securely hold the meshes in place. The mesh holders are controlled by a set of x, y, and z micromanipulators mounted on a lab jack adjacent to the stage (Figure 1b). The micromanipulators are used to position the sprayer and meshes such that the sprayer is normal to the meshes and 0° relative to the MS inlet. To aid in positioning, the lower mesh holder rests against an aluminium alignment bar (Figure 1b inset) that is affixed to the TMⁿ-DESI stage with a pair of clamps (location shown by dotted lines in Figure 1b). The alignment bar ensures that the mesh-to-inlet angle and distance remains constant when analyzing multiple samples located at different positions on the same mesh (different samples are accessed by motion in the y-z plane). The inter-mesh distance and M2-to-inlet distances are shown in Figure 1a.

Mass Spectrometry

In a typical TM²-DESI experiment, the meshes are first washed with a 1:1:1 mixture of water (H₂O), methanol (CH₃OH), and acetone (C₃H₆O) for 10 s and then air-dried. Each mesh is then secured to a holder via the rotatable posts. Using a micropipette, microliter volumes of catalyst and substrate are deposited on M1 and M2, respectively, and allowed to air-dry. The mesh holders are then attached to the micromanipulators while being careful to avoid contact between the meshes or with other surfaces. The mesh holders are adjusted to set the inter-mesh distance in the range of 2 mm-4 mm. Shorter M1-M2 distances have higher desorption/ionization efficiency for 2 deposited on M2, with 2 mm typically used for experiments (Supplementary Figure S1b). This is supported by COMSOL Multiphysics (ver. 4.4.0.248; COMSOL, Inc., Burlington, MA, USA) laminar flow simulations showing that N₂ velocity decreases by ~20% after passing through M2 (Supplementary Figure S1b inset).

After setting the meshes and sprayer in desired positions relative to the MS inlet, N_2 gas and liquid flow are initiated to generate a microdroplet spray containing a reagent such as oxidant *m*CPBA (Figure 1). Detection of $[2+H]^+$ from M2 indicates a linear dynamic range of approximately one to two orders of magnitude and signal intensity scales linearly with liquid flow rate (Supplementary Figure S1c and d). We hypothesize that impact of the primary microdroplets on M1

initiate reactions in desorbed secondary microdroplets generating active catalytic species that interact with the substrate upon impacting M2. Catalytic conversion of the substrate occurs in desorbed tertiary microdroplets as they travel towards the mass spectrometer. Unless specified otherwise, the Orbitrap MS is typically operated using the following parameters for full-scan mass spectra: m/z range=250–1500, resolution=100,000 at m/z 400, mass accuracy=2–5 ppm, microscan=1, injection time= 500 ms; capillary voltage=275°C, tube lens voltage=110 V, and spray voltage=0 kV. Settings that are different for tandem MS (MS/MS) include: microscans=3, injection time=50, 500, or 1000 ms.

Results and Discussion

TM^{*n*}-DESI has the ability to monitor catalytic cycles (on- and off-path processes) and capture highly reactive intermediates. Iron porphyrins such as Fe-TPFPP chloride ([1+Cl]) are a class of biomimetic catalysts capable of a wide variety of oxidation reactions including C–H hydroxylation[41, 52, 53]. The mechanism of Fe-mediated hydroxylation involves activation of **1** by an oxidant such as *meta*-chloroperbenzoic acid (*m*CPBA) to produce a Fe peroxo intermediate **1a**, which undergoes homolytic and heterolytic cleavage to yield high-valent iron oxo catalytic intermediates **1b** and **1c**, respectively (Scheme 1a). The active catalytic species (**1b** and **1c**) can then hydroxylate organic compounds such as propranolol[54] (**2**, Scheme 1a) on the aromatic ring (**2b**), regenerating **1** to complete the catalytic cycle[41].

When [1+Cl] (5 µL of 5×10⁻⁵ M in CH₂Cl₂) is deposited on M1 and activated by mCPBA $(5 \times 10^{-5} \text{ M in } 4:1)$ CH₂Cl₂:CH₃OH) in the reagent spray (no ionization voltage applied; 200 PSI N₂ pressure), desorbed secondary microdroplets containing active catalyst species react with [2+HCl] (5 µL of 5×10⁻⁵ M in CH₂Cl₂) deposited on M2. Desorbed microdroplets from M2 were analyzed by Orbitrap MS to identify species 1 (m/z 1027.977), 2 (m/z 260.164), and **2b** $(m/z \ 276.159)$, indicating that the complete catalytic transformation of 2 can be observed on the TM²-DESI time scale (Figure 2a). Fe-TPFPP can hydroxylate other substrates such as dapoxetine by TM²-DESI-MS (Figure 2a inset), as well as activate catalysts such as Fe-TDFPP (Figure 2b) and Fe-TDCPP (Figure 2c), indicating that the technique is generalizable for studying chemical reactivity. Analysis of the activation of 1 by mCPBA using negative mode TM^{1} -DESI (Figure 2d) identifies the formation of 1b at m/z 1078.939 [1b+Cl]⁻ and m/z 1198.962 [1b+mCBA]⁻, corresponding to the homolytic catalytic pathway. To our knowledge, these results represent the first direct observation of the Fe-porphyrin homolytic catalytic pathway by MS (previous FT-ICR ESI-MS studies observed the radical cation iron oxo 1a) [42, 43].

Further analysis of acquired Orbitrap mass spectra indicate that the m/z values corresponding to **1a** (Figure 2d) and **1c** (Figure 2a) are isobaric with ligand oxidation degradation products (Scheme 1b)[41]. Oxidative degradation of porphyrin



Figure 2. Positive-mode TM^2 -DESI-MS of the hydroxylation of 2 by (a) 1, (b) Fe-TDFPP, and (c) Fe-TDCPP using oxidant *m*CPBA. Inset of (a) shows the hydroxylated products of 2 and dapoxetine. (d) Negative mode TM^1 -DESI-MS of the hydroxylation of 2 by Fe-TPFPP using oxidant *m*CPBA. (e) Positive-mode TM^1 -DESI-MS showing self-oxidation of Fe-TPP (Scheme 1b). (f) Reactivity of five Fe porphyrin catalysts towards aromatic and aliphatic oxidation of 2 by TM^2 -DESI-MS (green dotted line represents aliphatic oxidation by *m*CPBA). Structures: TPFPP (Scheme 1a), TDFPP (Figure 2b), TDCPP (Figure 2c), TMP (Figure 2f), and TPP (Scheme 1b)

ligands can produce species having opened rings and containing oxygen atoms in various functional forms, as exemplified by reacting Fe-TPP (nominal $\Delta m/z=360$ compared with Fe-TPFPP) 3 (deposited on M1) with mCPBA (reagent spray) yielding structures 3a-3c (Scheme 1b). The structures of these TPP degradation products are in agreement with previous studies employing ultraviolet-visible spectroscopy and MS[55]. For Fe-TPFPP, the [TPFPP+O-H]⁻ (m/z 989.044; Supplementary Figure S2) degradation product is observed when free TPFPP ligand ([TPFPP-H]⁻ at m/z 973.051; 5 µL of 5×10⁻⁴ M in CH_2Cl_2) and [1+Cl] (5 µL of 5×10^{-4} M in 4:1 CH₂Cl₂:CH₃OH) are deposited at the same spot and then reacted with *m*CPBA in TM¹-DESI (there is a 10-fold increase in the signal compared with the absence of [1+Cl]; Supplementary Figure S2). These results clearly indicate that [1+Cl] undergoes self-oxidation. In addition to being isobaric with ligand oxidation products, species 1a is estimated to have a lifetime on the order of tens of microseconds [56], [see Supporting Information (SI)], for the calculation), which is significantly shorter than the estimated reaction time of TMⁿ-DESI (based on the N₂ velocity and DCIP estimations discussed below). As a result, ion signals at m/z1233.929 and 1353.951 are unlikely to contain the Fe-peroxo intermediate. However, observation of both 1b and 2b combined with the higher reported reactivity of 1c towards hydroxylation of 2[41] suggest that the peak at m/z 1043.972 possibly also contains the high-valent radical cation Fe-oxo 1c.

TM²-DESI provides a simple approach for separating reagents and controlling their order of introduction into the microdroplet spray. These capabilities are useful for step-wise elucidation of reaction mechanisms and for minimizing offpath processes. For example, mCPBA can oxidize 2 without catalyst interaction to generate 2a (aliphatic chain oxidation: green dotted line in Figure 2f), which is isobaric with catalytic product **2b** (aromatic oxidation)[41]. Species **2a** and **2b** were distinguished using MS/MS experiments (see SI; Supplementary Figure S3). When a premixed solution of [2+HCl] $(2.5 \times 10^{-6} \text{ M})$ and mCPBA $(2.5 \times 10^{-5} \text{ M})$ in 4:1 CH₂Cl₂:CH₃OH was sprayed towards the mass spectrometer at 30 μ L/min (no ionization voltage applied), a peak at m/z276.160 ($[2a+H]^+$) is observed at ~4% intensity relative to m/z $260.165 ([2+H]^{+})$, indicating aliphatic oxidation in the absence of the catalyst (Supplementary Figure S3). However, when [2+ HCl] (5 μ L of 5×10⁻⁵ M in CH₂Cl₂) is deposited on a mesh and analyzed using a microdroplet spray containing mCPBA $(5 \times 10^{-5} \text{ M})$, the relative intensity of $[2a+H]^+$ is ~0.06%, indicating a reduction in the off-path process by a factor of ~60. When a mesh bearing [1+Cl] (5 μ L of 5×10⁻⁵ M in CH₂Cl₂) was placed in front of the mesh bearing 2, the peak at m/z 276.160 had relative intensity of ~0.8% compared with $[2+H]^+$. So, when TM²-DESI was used to separate reagents and specify their order of introduction, the off-path process was reduced and the ion population at m/z 276.160 primarily represented on-path product 2b.

It is essential to determine the reactivity of ligands with different chemical motifs in the discovery and development of new catalysts. TM^{*n*}-DESI allows rapid simultaneous



Figure 3. (a) TM¹-DESI-MS of the reaction between *L*-AA and DCIP. (b) DESI mass spectrum of DCIP reduction by ascorbic acid. (c) Continuous-flow ESI-MS of of *L*-AA reacting with DCIP (reaction time= \sim 7 s)

characterization of reaction mechanisms and reaction progress. Depositing simultaneously five Fe catalysts with different porphyrin ligands on M1 and substrate 2 on M2, allowed rapid comparison of the relative vields of aliphatic and aromatic hydroxylation products in a single experiment (Figure 2f and Supplementary Figure S3; ratio of aliphatic to aromatic oxidation determined by MS/MS as described in the SI). The observed reactivity trend agrees with previously published reports showing that oxidizing power decreases with increasing electron density at the metal centre [41], suggesting that TM²-DESI-MS also provides information relevant to bulk solutionphase chemistry. Other spray-based ionization sources (e.g., continuous-flow ESI, stopped-flow ESI, and DESI) would require additional tubing, pumps, microdroplet emitters, or valves to achieve high-throughput analyses of complex reactions similar to TMⁿ-DESI-MS.

In addition to 2, dapoxetine (Figure 2a inset) and 1naphthyl-3-pyrrolidinyl (Supplementary Figure S4), which share an oxynaphthyl moiety, as well as four other substrates were successfully hydroxylated using Fe-TPFPP as the catalyst (structures of 1-naphthyl-3-pyrrolidinyl, rhodamine B, rhodamine 6G, fluorescein, and lauric acid shown in Supplementary Figure S4). A spray voltage of -5 kV was applied (Bertan 205b high voltage power supply, Spellman, Hauppauge, New York, USA) for TM²-DESI-MS oxidation of fluoroscein and lauric acid to improve sensitivity (the effect of spray voltage on signal intensities is discussed later in this article). Product-to-substrate ratios (P:S) calculated from the single-stage MS experiments indicate that the substrates containing aromatic rings are more easily oxidized by Fe-TPFPP compared with alkyl compounds. The location of the hydroxyl group was determined using MS/ MS (Supplementary Figures S5–S9).

TM²DESI characterization of the Fe-TPFPP reactions described above were performed without applying voltage to the spray solutions in order to closely mimic bulk-phase reaction conditions, except for fluorescein and lauric acid, which required -5 kV for adequate sensitivity. Application of 5 kV (positive and negative modes) in TM¹-DESI-MS characterization of Fe-TPFPP activation by mCPBA (deposited 5 µL of 10⁻⁴ M Fe-TPFPP in CH₂Cl₂; DESI microdroplet spray contains 10⁻⁵ M mCPBA in 4:1 CH₂Cl₂:CH₃OH) increased absolute signal intensities without significantly altering relative intensities (Supplementary Figure S10; viz., applying a voltage increased sensitivity). In addition, ion signals suggesting formation of reactive oxygen species [57] or other unwanted byproducts were not observed (Supplementary Figure S10). Experiments carried out at different capillary inlet temperatures, 225°C (Supplementary Figure S11a), 275°C (Figure 2d), and 325°C (Supplementary Figure S11b) in negative mode (0 kV) show that the relative intensities change, indicating effects on desolvation/ionization processes and/or reaction kinetics. Ion signals for $[1+2C1]^{-}$ (*m/z* 1097.916), $[1b+mCBA]^{-}$ $(m/z \ 1198.963)$, and $[1+Cl+mCBA]^{-}$ $(m/z \ 1217.938)$ increased relative to $[1+2mCBA]^{-}$ (m/z 1337.959). Increasing the capillary inlet temperature did not yield any new ion signals indicative of unwanted side reactions. All these observations

suggest that applying standard ionization voltages and temperatures in TMⁿ-DESI does not significantly influence the observed reaction pathways in acquired mass spectra for this catalytic system, thereby demonstrating the ability of TM-DESI to provide information relevant to bulk-phase chemistry.

TM^{*n*}-DESI has the ability to probe reactions on time scales less than 1 ms. When *L*-AA[58] (2×10^{-3} M in 1:1 CH₃OH:H₂O) was sprayed (5 µL/min; 200 psi nebulizing gas; 0.6 cm and 1.0 cm emitter-to-inlet distances; +3 kV) towards a mesh bearing DCIP (**4a**; 5 µL of 5×10^{-3} M in CH₃OH) in TM¹-DESI-MS, [**4b**+H]⁺ was observed at *m*/*z* 270.008 (Figure 3a) with intensity of ~4% relative to [**4a**+H]⁺ at *m*/*z* 267.993. Assuming that the concentration of DCIP in the secondary microdroplets is low compared with *L*-AA, the observed reaction time in microdroplets (*t*) for TM¹-DESI (t_{TM}) and DESI (t_{DESI}) can be approximated using *pseudo*-first-order kinetics [58] (see SI)

$$t = \ln\left(\frac{I_{4a} + I_{4b}}{I_{4a}}\right) / \left(k_f \cdot [AA]_0\right)$$

where k_f is the rate constant, $[AA]_0$ the initial L-AA concentration, I_{4a} the intensity of $[4a+H]^+$, and I_{4b} the intensity of $[4b+H]^+$. For DESI (Figure 3b), L-AA (2× 10^{-3} M in 1:1 CH₃OH: H₂O) was spraved (5 μ L/min; 200 PSI nebulizing gas; 0.6 cm and 1.0 cm emitter-toinlet distances; +3 kV applied) towards a paper surface containing DCIP (deposited 5 μ L of 5×10⁻³ M in CH₃OH). The ratio $[t_{DESI}/t_{TM}] \approx 2-3$ (Supplementary Table S1), which suggests that t_{TM} and t_{DESI} have the same order of magnitude of less than 1 ms (t_{DESI} was experimentally estimated by Latour and coworkers [59, 60]). These results agree with previous studies proposing that TM-DESI and DESI have relatively similar desorption/ ionization mechanisms [34], calculations showing that $[t_{ESI}]$ t_{TM} >40 at various emitter-inlet distances and concentrations (Supplementary Table S1), and calculations showing that microdroplets of TM²-DESI travel at twice the velocity of droplets generated with DESI; see Electronic Supplementary Information for the calculations) [45].

In this report, we describe an ambient ionization source that involves directing a microdroplet spray through a series of micrometer-sized meshes bearing reagents, referred to as multistage reactive transmission mode desorption electrospray ionization. This technique provides a simple approach for separating reagents and specifying their order of introduction into the microdroplet stream, which facilitates the step-wise analysis of complex catalytic reactions. In addition, the short travel time of the microdroplet spray from the emitter to the mass spectrometer inlet provides access to short reaction time scales less than 1 ms.

Acknowledgments

The Perry Research Laboratory gratefully acknowledges financial support from the University of Illinois at UrbanaChampaign (UIUC). T.J.C. thanks the National Science Foundation Graduate Research Fellowship, National Institutes of Health Chemistry-Biology Interface Training Program (NIH T32 GM070421), and UIUC Springborn Fellowship for financial support. The authors also acknowledge the work of Asenath Francis (Louisiana State University; funded by the NSF Research Experience for Undergraduates program at UIUC) for characterizing the oxidation of various substrates. Kevin Parker (UIUC undergraduate) was instrumental in early studies characterizing reactions and ion source configurations. The authors acknowledge Jedidiah Veach (UIUC graduate student) for help in designing the continuous-flow ESI-MS figure located in the SI. We also thank the staff of the UIUC School of Chemical Sciences Machine Shop for their expertise in designing and machining ion source components.

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