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Identification of Fleeting Electrochemical Reaction Intermediates Using Desorption Electrospray Ionization Mass Spectrometry

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Supporting Information

ABSTRACT: We report a new method for the mass spectrometric detection of fleeting reaction intermediates in electrochemical reactions utilizing a "waterwheel" working electrode setup. This setup takes inspiration from desorption electrospray ionization (DESI) mass spectrometry, where the sampling time is on the order of milliseconds, to sample directly from the surface of a working electrode for mass spectrometric analysis. We present data that show the formation of a diimine intermediate of the electrochemical oxidation of uric acid that has a lifetime in solution of 23 ms as well as data that provide evidence for the formation of a similar diimine species from the electrooxidation of xanthine, which has not been previously observed.

In situ analysis of electrochemical reactions is traditionally carried out using cyclic voltammetry,[1] spectroelectrochemistry,[2,3] and/or surface scanning probe techniques.[4] However, these methods lack high chemical specificity. In this regard, mass spectrometry (MS) can serve as a powerful detector for electrochemical reactions with high sensitivity and selectivity because MS can provide molecular weight information for products and/or intermediates of redox reactions.[5−8]

Online coupling of electrochemistry (EC) with MS has a history of over 40 years[9] that includes ionization techniques such as electrospray ionization (ESI),[6,10,11] desorption ESI (DESI),[7,11] thermospray,[12−14] nanoDESI,[15] and differential electrochemical mass spectrometry (DEMS).[16,17] The challenge in acquiring faster response times exists in transferring electrochemically generated species quickly from solution to the gas phase for MS detection. DEMS has been shown to have response times as fast as 0.1 s,[17] but most electrochemistry coupled ambient ionization mass spectrometry methods typically have response times on the order of seconds.[18,19] The development of DESI[20−22] and the subsequent easy ambient sonic-spray ionization (EASI),[23−25] allows analyses to be made on surfaces under atmospheric pressure at room temperature with a sampling time on the order of milliseconds.[26,27] The technique presented below is an ambient ionization mass spectrometric approach that utilizes DESI-MS with no high voltage applied to the spray solution. The electrode surface is sampled in situ by fast transfer of electrogenerated species to the gas phase for analysis by the mass spectrometer on the time scale of milliseconds.

As depicted in Figure 1, the experimental design employs a round rotating platinum working electrode with a width of 0.64 cm (0.25 in) and a diameter of 3.81 cm (1.50 in) that is partially immersed in an aqueous electrolyte solution. As the working electrode rotates, a thin layer of liquid film (approximately 1 mm thick) develops on the electrode surface, forming a "waterwheel". A platinum wire counter electrode and a Ag/AgCl reference electrode are immersed in the reservoir of electrolyte solution. A metal contact (not shown) rests against the platinum working electrode to complete the three-electrode system. Above the rotating waterwheel system, a custom spray probe directs a spray of analyte microdroplets to the surface of the working electrode. To ensure that any electrochemical

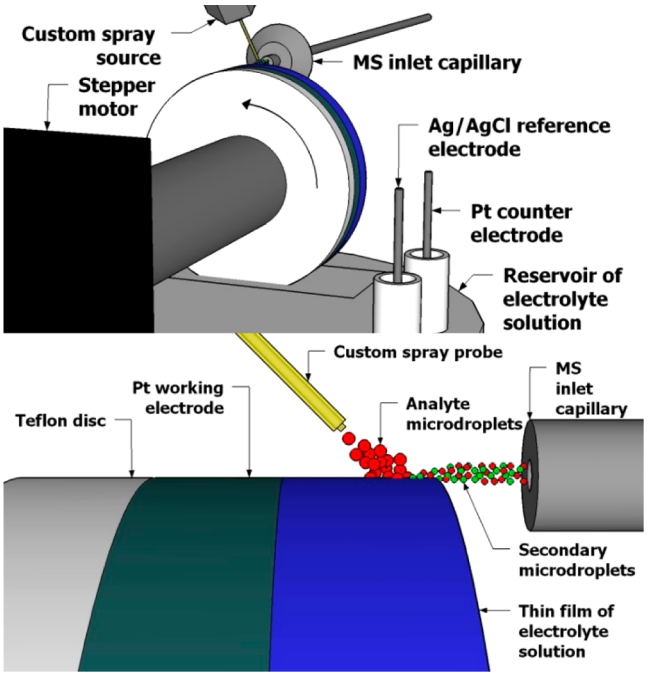

Figure 1. (top) Apparatus used to detect electrochemical reaction intermediates, employing a rotating waterwheel electrode. The direction of rotation is indicated by the arrow. (bottom) Zoomed-in area showing sample ionization.

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oxidation or reduction is constrained to take place at the electrode surface, no high voltage is applied to the analyte spray. The spray droplets hit the surface of the thin film of electrolyte solution, which is now thinner than 1 mm because of the N_2 nebulizing gas pressure, on the surface of the working electrode. Tinier secondary microdroplets are directed into the mass spectrometer[28,29] and are analyzed with an LTQ Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific, San Jose, CA) where the m/z ratio is determined utilizing the high mass accuracy and high resolving power of the Orbitrap mass analyzer.[30] A potentiostat (WaveNow, Pine Research Instrumentation, Durham, NC) is used to apply a potential across the three electrodes. The electrolyte solution in the bath is 1 mM ammonium acetate unless specified otherwise.

The electrochemical oxidation of triphenylamine (TPA) is well-understood,[18,31,32] so TPA was chosen as a proof-of-concept analyte. A 100 μM TPA solution was prepared in 1:1 MeCN/H_2O with 1% acetic acid. When the analyte solution is sprayed at an infusion rate of 5 μL/min at the surface of the rotating waterwheel electrode rotating at 2.5 rev/s, a cation at m/z 246.1266 is observed, which is ascribed to protonated TPA (Figure 2a; theoretical m/z 246.1277, error −4.5 ppm).

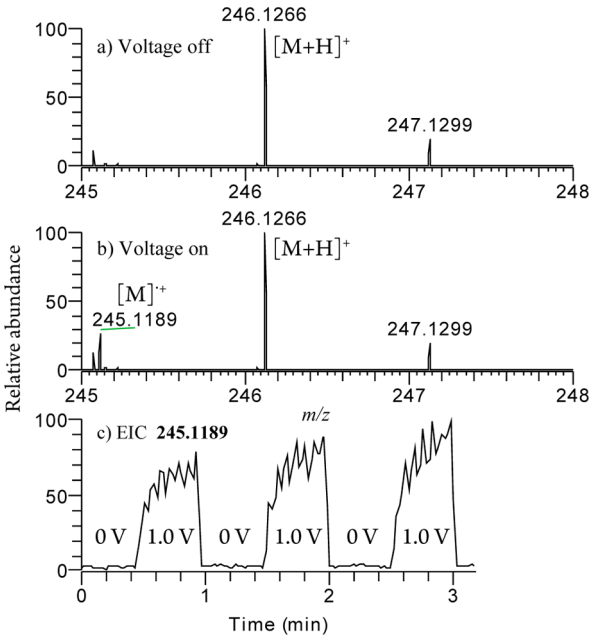
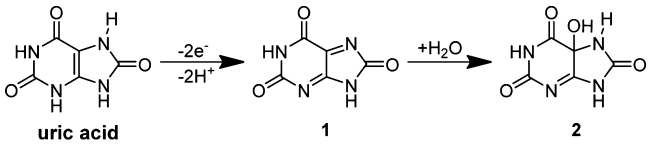


Figure 2. (a, b) Positive-ion-mode mass spectra of triphenylamine with (a) 0.0 V and (b) 1.0 V applied to the working electrode. (c) EIC for the m/z 245.1189 peak as a function of the applied potential.

Applying an oxidation potential of 1.0 V to the rotating working electrode results in the formation of an ion at m/z 245.1189, which is attributed to the TPA radical cation as a result of electrochemical oxidation of TPA (Figure 2b; theoretical m/z 245.1199, error −4.1 ppm). The signal intensity of the radical cation increases greatly when an oxidizing potential is applied to the rotating working electrode relative to when no potential is applied to the rotating working electrode, as is observed in the extracted ion chromatogram (EIC) of the m/z 245.1189 peak (Figure 2c). The high mass accuracy and the signal response of the m/z 245.1189 peak to an oxidation potential provides strong evidence for the formation of the radical cation by way of electrochemical oxidation of TPA. Peaks associated with dimer species are observed that have been previously reported,[18,31,32] corresponding to the radical cation dimer at m/z 488.2241 (theoretical m/z 488.2247, error −1.2 ppm) and the dicationic dimer at m/z 244.1122 (theoretical m/z 244.1121, error +0.3 ppm). The signal intensity of each dimer peak markedly increases when an oxidizing potential is applied to the working electrode relative to when no potential is applied, indicating that these species are also formed as a result of TPA electrooxidation (see the Supporting Information). TPA electrochemical oxidation as a function of potential at different electrode rotation rates has also been studied, and the results are presented in the Supporting Information.

With the success of TPA electrochemical oxidation, the next goal was to investigate the feasibility of using this experimental setup to observe transient electrochemical reaction intermediates. Uric acid and xanthine, two biologically important molecules, were chosen for investigation. It is known that the electrochemical oxidation and enzymatic oxidation of uric acid and a number of its derivatives proceed by similar reaction pathways.[33−35] The proposed mechanism for the oxidation of uric acid at physiological pH initiates with a two-electron, two-proton ($2e^-$, $2H^+$) oxidation, leading to the formation of an unstable intermediate proposed to be a diimine species (Scheme 1, **1**) on the basis of cyclic voltammetry.[36] This

Scheme 1. Initial Steps of Uric Acid Oxidation



diimine species, with a measured half-life in solution of 23 ms at pH 8, readily reacts with H_2O to yield the imine alcohol, whereas at higher or lower pH the half-life of the diimine species is even shorter.[33] Previous EC-MS studies have not reported the observation of the diimine species in situ, but the formation of the imine alcohol (Scheme 1, **2**) has been observed.[13,35] The proposed diimine intermediate was observed with MS when the imine alcohol was fragmented via tandem MS,[35] but the diimine intermediate has not been observed using online EC-MS techniques.

A 100 μM solution of uric acid in water with 1% acetic acid was prepared. When the analyte solution is sprayed at an injection flow rate of 15 μL/min onto the working electrode rotating at 2.5 rev/s, the deprotonated uric acid anion is observed at m/z 167.0209 (Figure 3a; theoretical m/z 167.0211, error −1.2 ppm). When an oxidation potential of 1.0 V is applied across the rotating working electrode, a peak at m/z 165.0053 is observed, which is ascribed to the diimine intermediate (Figure 3b; theoretical m/z 165.0054, error −0.9 ppm). The MS signal of this species increases greatly when an oxidizing potential is applied to the working electrode, as is observed in the EIC (Figure 3c). This observation indicates that it originates from the electrolysis of uric acid. Interestingly, the imine alcohol **2** (theoretical m/z 183.0160) was not observed to have a correlation with the application of the oxidation potential, emphasizing the fast transfer of electrochemically generated species from the electrode surface to the mass spectrometer. Previous work utilizing this waterwheel setup has been performed in which the imine alcohol has been observed (see the Supporting Information). This is the first

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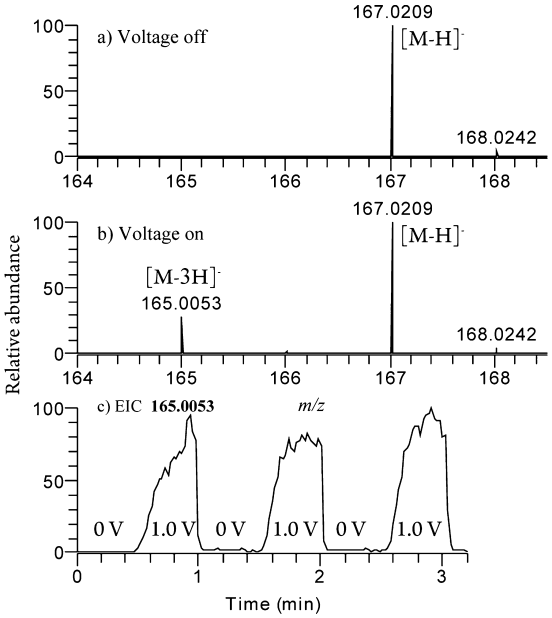


Figure 3. (a, b) Negative-ion-mode mass spectra of uric acid with (a) 0.0 V and (b) 1.0 V applied to the working electrode. (c) EIC for the m/z 165.0053 peak as a function of the applied potential.

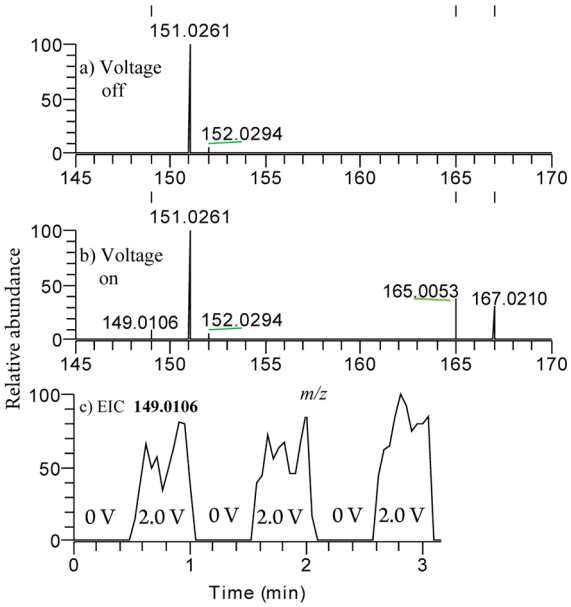


Figure 4. (a, b) Negative-ion-mode mass spectra of xanthine with (a) 0.0 V and (b) 2.0 V applied to the working electrode. The peak regions for m/z 149.0106, 165.0053, and 167.0210 have been magnified 50 times for clarity. (c) EIC for the 149.0106 peak as a function of the applied potential.

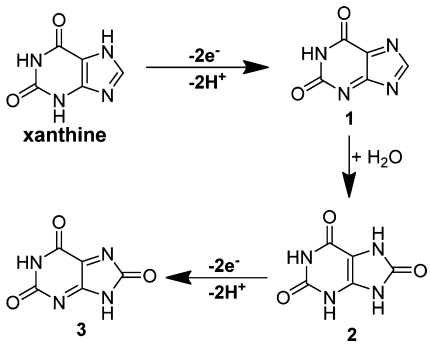
online EC-MS observation of the unstable diimine, which provides strong evidence for the formation of the diimine intermediate in the electrochemical oxidation of uric acid.

Xanthine, another purine compound, was also examined. Xanthine is proposed to undergo a series of electrochemical oxidations, the first giving uric acid and the subsequent ones following the oxidation pathway of uric acid.[33,36] Unlike uric acid, a diimine species produced from the direct $2e^-$, $2H^+$ electrooxidation of xanthine has not been observed by EC-MS or cyclic voltammetry, leading to a proposed electrooxidation mechanism from xanthine directly to uric acid.[33,36]

A 1 mM xanthine solution was prepared with 10 mM ammonium acetate in water and 0.8% acetic acid. When the analyte solution is sprayed at an injection flow rate of 20 μL/min onto the working electrode rotating at 1.5 rev/s, the deprotonated xanthine is observed in negative-ion mode at m/z 151.0261 (Figure 4a; theoretical m/z 151.0261, error −0.3 ppm). When an oxidation potential of 2.0 V is applied to the rotating working electrode, a peak at m/z 149.0106 is observed (Figure 4b), ascribed to the xanthine diimine (Scheme 2, **1**, theoretical m/z 149.0105, error +0.5 ppm); this peak is observed only when an oxidation potential is applied to the working electrode (Figure 4c). This is the first online EC-MS evidence for the formation of a xanthine diimine intermediate in the electrochemical oxidation of xanthine.

Upon the application of an oxidation potential to the working electrode, peaks were also observed at m/z 167.0210, attributed to the water addition product to form uric acid (Figure 4b; theoretical m/z 167.0211, error −0.4 ppm), and m/z 165.0054, attributed to species **3** of Scheme 2, the $2e^-$, $2H^+$ electrooxidation product of uric acid (Figure 4b; theoretical m/z 165.0054, error −0.3 ppm). The MS signal intensity of each of these species increases greatly with the applied potential, indicating that they originate from the electrochemical oxidation of xanthine (see the Supporting Information). Comparison of Figures 3b and 4b shows that the same m/z 165 and 167 ions observed for the electrochemical oxidations of

Scheme 2. Initial Steps of Xanthine Oxidation



both uric acid and xanthine. This supports the proposed mechanism that xanthine oxidizes to uric acid before undergoing further oxidation along the uric acid oxidation pathway.[36]

This study has presented a new application of electrochemistry coupled with ambient ionization mass spectrometry that is capable of detecting transient intermediates generated on the electrode surface. The transient diiimine intermediates resulting from electrochemical oxidations of uric acid and xanthine were successfully captured and analyzed by MS. Because of the sensitivity of MS, minute amounts of analyte are needed for this experiment. The mass information offered by MS gives this approach high specificity. The results show promise for capturing active electrochemical intermediates on an electrode surface by employing a rotating waterwheel electrode cell with fast transfer of electrogenerated species from the electrode surface to the gas phase for MS detection. This ambient ionization mass spectrometric technique probes electrochemical reactions on the time scale of milliseconds, which can elucidate mechanistic information for many oxidation−reduction reactions.

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■ ASSOCIATED CONTENT

Supporting Information

Mass spectra, text, and figures giving experimental details. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b03862.

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Notes

The authors declare no competing financial interest.

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