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14. ABSTRACT

A summary of three years of work is difficult to reduce to a few sentences. We have applied Hadamard transform mass spectrometry to stop-flow kinetics and shown how this technique can complement previous stop-flow studies that rely instead on spectroscopic signatures. We have also examined hypergolic reactions. In particular, we performed electrospray ionization mass spectrometry (ESI-MS) and MS/MS fragmentation studies to determine the composition of a previously unknown precipitate. We discovered that the precipitate consisted of melamine oligomers and polymers. However, the majority of the effort involved an ambient ionization method that can be carried out at atmospheric pressure at room temperature -- desorption electrospray ionization (DESI). Here charged droplets are fired at some surface and the resulting splash of smaller droplets enter a mass spectrometer. We applied this technique to show how to overcome adverse effects from salt and detergent in electrokinetic separations using a spinning disk. We also applied this technique, we believe with great success, to study reaction intermediates and to demonstrate the potential of using DESI for mass spectrometric imaging.

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FINAL REPORT

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1. Stopped-flow apparatus interfaced to Hadamard TOF

Hadamard TOF (HT-TOF) mass spectrometry analyzers are capable of sampling rates in excess of 1000 Hz, which are necessary in order to resolve kinetic studies at low timescales. In progress towards this goal, we interfaced a stopped-flow apparatus consisting of a modified sampling valve with an electrospray ion source. Using the incredible time resolution of the HT-TOF, we were able to determine the minimum reaction time of the stopped-flow apparatus as 100 ms and we also followed the progress of a tryspin catalyzed peptide digestion with about 1 ms time resolution (1).

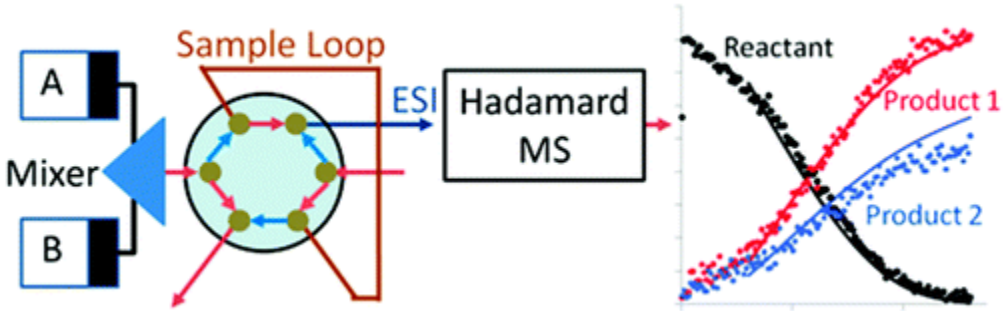


Fig.1: Diagram of Stopped-Flow Apparatus and Representative Reaction Monitoring Experiment

2. Using Desorption Electrospray Ionization Mass Spectrometry to detect transient intermediates and decomposition products of catalyzed organometallic reactions

Identifying intermediates is essential to understanding chemical reactions. However, few analytical techniques can intercept transient species in solution because of their low concentrations, short lifetimes, concurrent side reactions, and the complexity of the reaction environment. The high speed, sensitivity, and chemical resolution of mass spectrometry (MS) make it one of the preferred methods for studying reactivity.

2.a) Interfacing micellar electrokinetic chromatography to MS

Salts and detergents used in the mobile phase for electrokinetic separations suppress ionization efficiencies and contaminate the inlet of the mass spectrometer. In this project, we developed a new method that uses desorption electrospray ionization to overcome these limitations. Effluent from capillary columns is deposited on a rotating Teflon disk that is covered with paper. As the

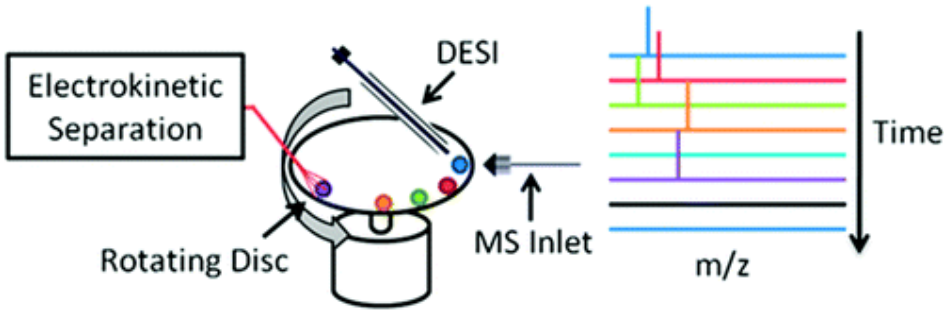


Fig.2: Scheme of DESI on a rotating disk

surface rotates, the temporal separation of the eluting analytes (i.e., the electropherogram) is spatially encoded on the surface. Then, using DESI, surface-deposited analytes are preferentially ionized, reducing the effects of ion suppression and inlet contamination on signal. We demonstrated the use of this novel approach with two capillary-based separations: a mixture of the rhodamine dyes at milligram/milliliter levels in a sodium borate solution was separated by capillary electrophoresis, and a mixture of three cardiac drugs at milligram/milliliter levels in a sodium borate and sodium dodecyl sulfate solution was separated by micellar electrokinetic chromatography. In both experiments, the negative effects of detergents and salts on the MS analyses were substantially minimized (2).

2.b) In situ catalyst synthesis and intermediate detection

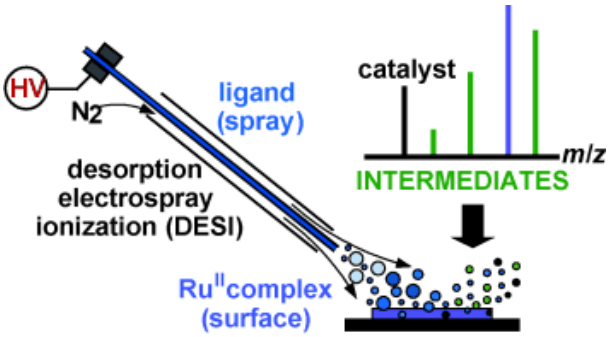


Fig.3: Schematic of Reactive DESI-MS Process

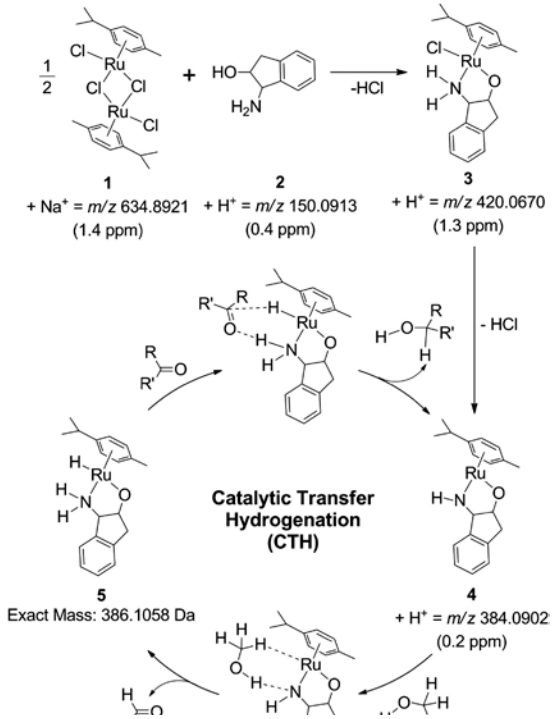


Fig.4: Detected Intermediates and Proposed Mechanism

Using desorption electrospray ionization mass spectrometry (DESI-MS) we were able to prepare the active transfer hydrogenation catalyst (3, Fig.4) on the millisecond timescale and initiate a transfer hydrogenation reaction in the droplets of a DESI spray. Intermediates were intercepted and identified by mass-to-charge ratio, exact mass, and, when available, MS/MS fragmentation. This work has been summarized in two publications (3) and (4).

This work includes the first use of DESI-MS to accomplish millisecond timescale mechanistic studies on catalytic reactions, which we believe will be a useful tool for studying and detecting intermediates in fast, solution-phase reactions.

2.c) Capturing Fleeting Intermediates in a C-H Amination Reaction Cycle

Recently, the Zare laboratory demonstrated that desorption electrospray ionization can intercept catalytic intermediates with millisecond time resolution. In DESI, primary microdroplets bombard a surface of interest, extracting physisorbed chemicals into secondary microdroplets (Fig. 5). The secondary microdroplets evaporate, producing dry ions that enter the mass spectrometer. By placing reagents in the spray and on the surface, chemical reactions occurring in the secondary microdroplets can be analyzed in real-time. Because of the short travel time from the surface to the mass spectrometer inlet, it is possible to intercept species formed during the first few milliseconds.

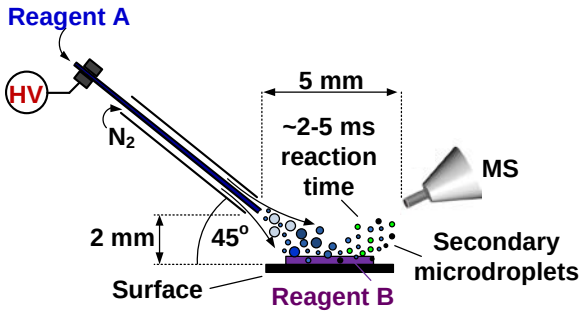


Fig.5: Experimental setup that used DESI to intercept reaction intermediates in solution on the millisecond timescale. Velocity of the droplets exiting the sprayer = ~100 m/s; Velocity of the secondary microdroplets = 4 m/s.; Reaction time = ~2-5 ms

Using this approach, our lab has made a significant discovery: The detection of all the proposed major intermediates (**1**-**7** in Fig. 6) in C–H aminations catalyzed by the dirhodium tetracarboxylate complex $Rh_2(esp)_2$ (**1**). Perhaps one of the most amazing results of this study is observation of the Rh-nitrene **6**, which has never been previously observed because it is postulated to have a half-life in the nano- to microsecond regime (5). These results highlight the remarkable power of DESI-MS to intercept fleeting intermediates in solution. We are also developing new mass spectrometric instrumentation to facilitate real-time reaction monitoring of air-sensitive catalysts and for probing reactivity in various gaseous environments. These technologies will significantly facilitate new discoveries in organometallic catalysis.

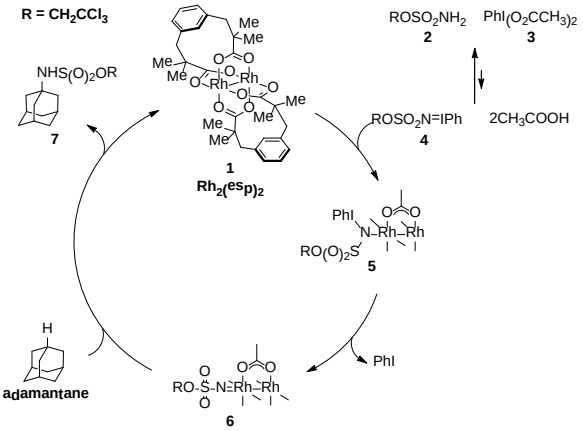


Fig.6: Proposed mechanism for $Rh_2(esp)_2$-catalyzed C-H aminations

2.d) Condensed phase mechanism studies of hypergolic reactions involving dicyanamide ionic liquids

Hypergolic fuels, or hypergols, can be ignited by exposure to an oxidizing agent under ambient conditions and are a common fuel source. Many hypergols are toxic, corrosive, and/or volatile such that they are difficult to handle and harmful to the environment. Dicyanamide (DCA) based ionic liquids are a less volatile alternative that are less viscous than most ionic liquids; however, ignition of these compounds results in incomplete combustion and produces a significant amount of precipitate. We performed electrospray ionization mass spectrometry (ESI-MS) and MS/MS fragmentation studies in order to determine the composition of this previously unknown precipitate. We discovered that the precipitate consisted of melamine oligomers and polymers. Interestingly, by adding the oxidant as a dilute solution to avoid ignition, oxidation of DCA liquids proved to be a facile and ambient synthesis of cyclic azines (6).

2.e) Identifying catalyst arrest mechanisms in a Ruthenium-catalyzed hydroxylation reaction

The goal of this (ongoing) project is to identify the active catalytic species of the hydroxylation reaction and to identify possible decomposition products of the catalyst. We made significant progress in achieving these goals in the last year. Looking at the reaction at the millisecond timescale (DESI-MS) we found the substrate, a small amount of product, and we identified a monooxo- as well as a dioxo-Me3tacnRu-species (Fig.7). In incubated mixtures we found various catalyst-adducts, as well as dimers and trimers. Further, we found that the composition of the reaction mixture changes with reaction/incubation time. Higher mass range signals are more likely to show up in longer reaction times, what could support the hypothesis that the efficiency of the reaction is limited by oligomerization of the catalyst. Ongoing investigations focus on the identification of the higher mass signals as well as the confirmation of the origin of catalyst adducts by labeling studies.

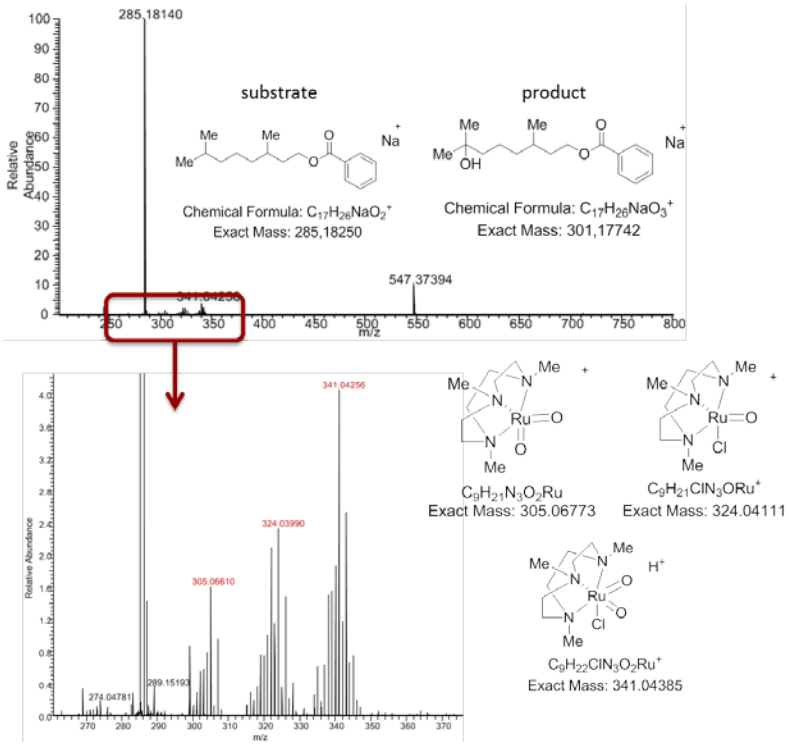


Fig.7: Identification of the active catalyst molecules in a ruthenium catalyzed hydroxylation reaction

3. Monitoring Catalytic Processes on Electrode Surfaces in Real-Time

After the exciting results from Perry et al(4) in 2012, the group sought to simplify the system to study electrocatalytic process on electrode surfaces. The aim of the new setup is to analyze the reaction during the catalytic process on the electrode surface instead of analyzing the catalyst species after the electrocatalysis. This required replacing the working electrode, counter electrode, and reference electrode with a smaller electrode setup. The small surface area and ability to utilize electrochemical processes made the screen printed electrode our first choice. Because the three electrodes of the system need to be in contact, desorption electrospray ionization would not be applicable. The analytical technique used is nanoDESI, where a liquid bridge connects two capillaries (Fig.8). One capillary feeds the sample to the electrode surface while the other feeds the mixture to the mass spectrometer. To test the nanoDESI electrochemical setup, the already charged molecule methyl viologen was used. The initial experiment sought to reduce methyl viologen to the methyl viologen radical (Fig.9). The change in the mass spectrum would show a decrease in the 93 m/z peak and an increase in the 186 m/z peak.

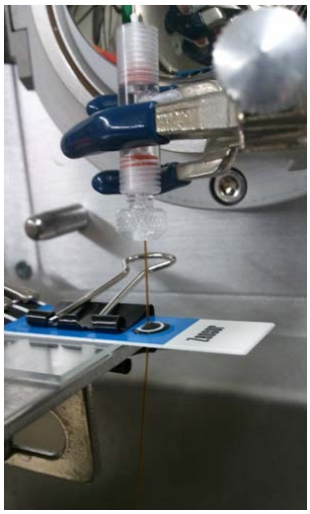


Fig.8: A picture of the nanoDESI setup using the screen printed electrode.

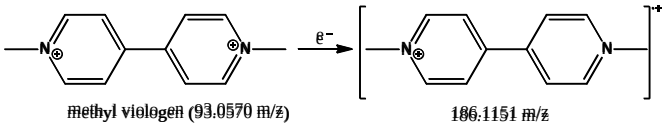


Fig.9: Reduction of methyl viologen to the methyl viologen radical.

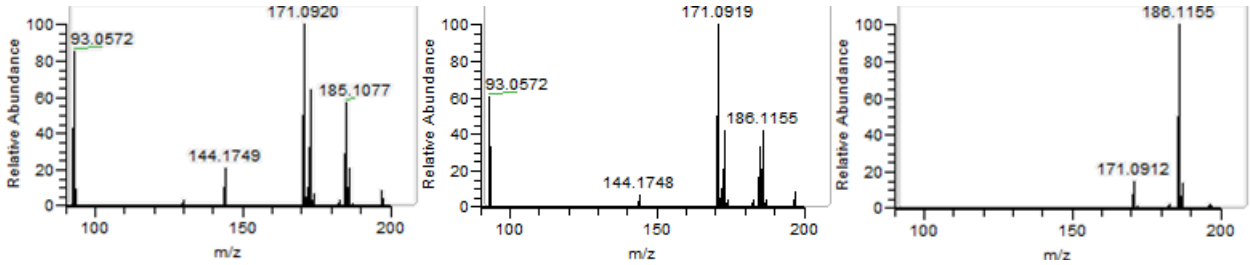
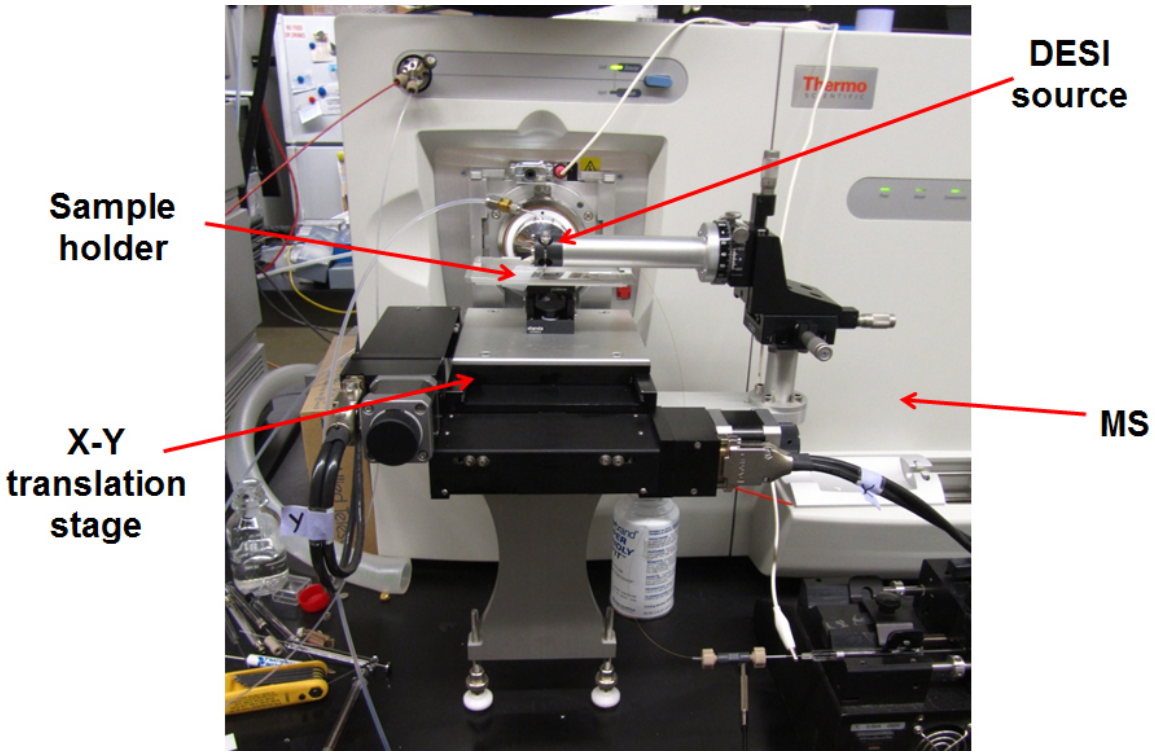


Fig.10: The initial results of the reduction of methyl viologen. The methyl viologen solution (left) before an applied potential, (center) -3V applied to the working electrode, and (right) the software subtraction of the two spectra.

The initial results show that the methyl viologen radical is formed after applying a negative potential to the solution (Fig.10). With these results, we believe that the nanoDESI setup with the screen printed electrode will perform the electrochemistry and the mass spectrometry analysis of the solutions in which an electrocatalyst is present.

4. Desorption Electrospray ionization Mass Spectrometric Imaging

By placing a sample on an XY translation stage, it is possible to make a spatial map of the chemical composition by choosing an appropriate solvent for the bombarding droplet in a DESI setup, as pictured below:



The resolution is limited to the size of the droplet and how well droplets can be aimed at the surface target. The present resolution is about 100 microns. The first applications were made to mouse liver tissue to map the chemical composition of lipid molecules in cancerous and noncancerous regions (7).

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