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Elusive Reaction Intermediates in Solution Explored by ESI-MS: Reverse Periscope for Mechanistic Investigations

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analytical methods · homogeneous catalysis · mass spectrometry · multicomponent reactions · reaction mechanisms

Just as periscopes allow a submarine to visually search for objects above the surface of the sea, in a reversed periscope fashion electrospray mass spectrometry (ESI-MS) can analyze the compounds at the gas phase/liquid phase interface for chemical entities which may exist in solution. The challenge is the identification and structural characterization of key elusive reaction intermediates in chemical transformations, intermediates which are able to explain how chemical processes occur. This Minireview summarizes recent selected publications on the use of ESI-MS techniques for studying solution intermediates of homogeneous chemical reactions.

1. Introduction

A pioneering work by Chen and co-workers on the use of electrospray ionization mass spectrometry (ESI-MS) as a key tool for screening challenging chemical entities and constrained organometallic complexes, like those formed in homogeneous catalysis, appeared in this journal almost two decades ago.[1a] Chen and co-workers, a few years later, published a paper wherein charged derivatization of substrates was used to sample intermediates and active species, of a catalytic reaction, by ESI-MS.[1b] Since then, quite a number of pivotal ESI-MS papers have been published on the investigation of the elementary steps of important chemical transformations.[2–4] In this context "soft" electrospray ionization can be considered a monitoring interface for ions and ionizable species existing in solution, thus conveying them into the gas phase for observation.

The potential of ESI-MS for this application is twofold:

1) The reaction mixture composition may be monitored over time as a result of the so-called source capability of "fishing out" ionic or ionizable intermediates from solution. In favorable cases, crucial species, with short life times, present at very low concentration can be efficiently transferred from the reaction solution to the gas phase, where they become immune to bimolecular reactions and thus are more conveniently observable.
2) The ionic or ionized intermediates, once isolated in the gas phase, can be structurally characterized and their reactivity investigated.

In principle all the reactions occurring in solution can be investigated by ESI-MS, including complex multicomponent reactions,[5] organometallic transformations,[6] polymerization processes, and inorganic reactions. Even oxygen- and moisture-sensitive reaction mixtures can be analyzed in situ without affecting the ongoing chemical process.[7] Moreover the applicability of the technique can be widened by appending ionic tags to the reactant, thus allowing non-ionizable species to be sampled from solution and studied.[8] The potential of this technique can be complemented and increased by MS^n (n: multiple steps of product ion analysis) performance, ion mobility, ion spectroscopy,[9] and computational studies.[10,11]

Herein we aim to review the most relevant and recent contributions on mechanistic studies of organic reactions, studies carried out by intercepting intermediates with ESI-MS. We will also address the reliability of the various methods, as well as their use in combination with the above-mentioned techniques.

2. Beyond the Scepticism

Historically ESI-MS investigations of reaction mechanisms have been considered less reliable than standard studies which are performed using the condensed phase. This bias is due to three major ESI-MS features: 1) the mass spectrometric analysis occurs in the gas-phase; 2) all species are sampled in a charged state; 3) ESI, even if considered a soft ionization technique, may alter the authentic composition of a complex reaction mixture.[7,11] Indeed, ionization processes which

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occur in the ESI source take place within the microdroplets generated by the application of a high voltage. These droplets, accelerated within a reduced-pressure region, are introduced to a flux of heated nitrogen which helps nebulization and solvent evaporation, thus keeping the overall process almost adiabatic. Only at the end of the process, close to the high-vacuum interface, can the desolvation process be considered complete, thus leading to naked ions in the gas phase. In the case of sampling an ongoing reaction, the charged and ionizable molecular species present in solution should be, in principle, fished out and the reaction intermediates are transferred to the gas phase.[2a,12] During the evaporation process, however, the solution is concentrated and the pH changes, thus possibly altering the nature of the reactive species present in the original sample.[4,13a] Although these considerations are absolutely valid and deserve to be carefully taken into account during mechanistic investigations using ESI-MS, it is without question that ESI-MS is able to reveal a significant portion of the species in solution.[2a,7,11,12] In particular, very recently, several protocols have been developed to prove the correlation between reaction intermediates existing in solution and the ions transferred to the gas phase in the mass spectrometer.[7]

2.1. Development of Efficient Real-Time Reaction Monitoring

Usually the time-dependent composition of an ongoing reaction mixture is investigated by offline ESI-MS analysis. This approach consists of sampling aliquots of a reaction solution at different times throughout the course of the reaction. Each of them is immediately diluted and analyzed by ESI-MS using direct infusion. Such a traditional method, however, does not give highly accurate data since it is challenging to evaluate the exact reaction time, especially when dilution of the sample is required. Moreover, the dead time required for sampling the reaction mixture and subsequent sample manipulation prevents the analysis from being carried out at the very beginning of the reaction when, for example, all the reactants are mixed. Better results have been achieved by online analyses using microreactors directly coupled with the electrospray source,[3] or other such set-ups.[14] These approaches greatly reduce the dead time before the analysis, and allow the study of reaction mixtures at fixed reaction times.

The breakthrough strategy for recording high-quality time-dependent spectra was developed by McIndoe and co-workers.[15] They introduced a simple pressurized sample infusion (PSI) system for ESI-MS, for the straightforward and continuous monitoring of a reaction mixture. Accordingly, when a small additional pressure is applied to a conventional Schlenk flask, the reaction mixture is continuously pumped to the ESI apparatus through a short-length PEEK (polyether ether ketone) capillary which is submerged in the solution (Figure 1). The over pressure can be provided by an inert gas, which also has the advantage of preventing alteration of air- and moisture-sensitive species, and in some cases, a gaseous reactant itself (even a gas-filled balloon can be used as a pressure source).[7,13] This system also allows monitoring of a reaction at any temperature below the boiling point of the solvent. The direct sampling approach proved to be particularly useful for organometallic chemistry. It also ensures fast and accurate kinetic data by tracking real-time growth and decay of intermediates associated with catalytic reactions.[16]

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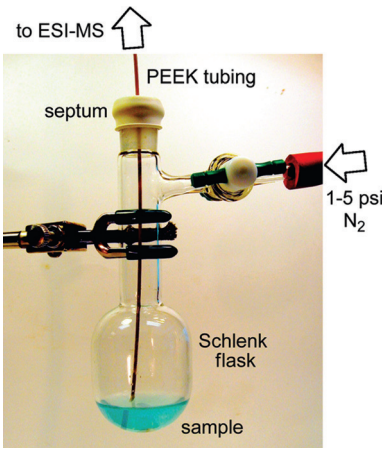


Figure 1. Setup for pressurized sample infusion ESI-MS, direct from a Schlenk flask.[15]

By using a similar apparatus, Cooks and co-workers obtained outstanding results from the real-time monitoring of three important reactions.[13a] Short-lived intermediates (ca. 5 s) were observed in Pd/C-catalyzed hydrogenolysis, and several intermediates were seen in both reductive amination and Negishi cross-coupling reactions.

2.2. Isotopic-Labelling Experiments

Isotopic labelling, in particular hydrogen–deuterium exchange (HDX), is key for elucidating the active structure of proteins, and also for studying protein–protein and protein–ligand interactions. Such a method is also used for studying reaction mechanisms in the gas phase (this application is beyond the scope of this Minireview). As to solution-phase reaction-mechanism studies, to the best of our knowledge, isotopic labelling is restricted to only two examples (see below). We consider these studies to be well-designed and they could conceivably pave the way to new applications.

ESI may create artifacts during the spraying process, with formation of undesired adducts which might not be present in the reaction solution.[7] To distinguish reaction intermediates present in solution from those species formed during the ESI process, one equivalent of an isotopically labelled reagent, which is supposed to take part in the formation of the intermediate, can be added to the ongoing reaction mixture. Soon after the addition only the unlabelled moiety is revealed as being part of the intermediate species, while after equilibration a 1:1 mixture of the labelled and unlabelled species is revealed. The observation of the time-dependent evolution of labelled and unlabelled intermediates is thus used to confirm the presence of the intermediate adducts in solution. Such an elegant approach was successfully employed by Roithová and co-workers when studying intermediates of gold-mediated alkyne oxidation[17] and gold-catalyzed addition of methanol to alkynes.[18] In both cases they added a deuterated reactant, namely using [D_5]pyridine *N*-oxide in the former study and CD_3OD in the latter which were added to a pre-stirred reaction mixture showing the MS signals for the intermediates of interest. They observed an equalization of the MS peaks over time with the peaks of the corresponding deuterated counterparts, and clearly assigned putative intermediates as species which were truly fished out from the solution. Moreover such isotopic-labelling experiments may give insights into the half-life of an intermediate in solution, at a certain reaction temperature.[18]

2.3. IRMPD Spectroscopy

Strictly speaking, the structural characterization of a ion in the gas phase by MS, conventionally achieved by collisional induced dissociation (CID) tandem MS, is an indirect speculative process based on measuring the composition of its product ions.[12b,19] Even though the chemical plausibility of such an inferred structure is generally accepted, the exact and complete atom connectivity within the ion of interest cannot be always achieved, especially for organometallic species and for high-energy reaction intermediates. Nowadays tracking such structural insight, which is of dramatic importance for mechanistic investigations, is feasible by taking advantage of infrared multiphoton dissociation (IRMPD) spectroscopy (Figure 2).[9]

Two European facilities, the Dutch FELIX[20] and the French CLIO,[21] have developed Fourier-transform ion cyclotron resonance (FTICR) mass spectrometers interfaced with free electron laser (FEL) infrared light sources. In this innovative apparatus, the tuneable FEL-generated infrared beam is directed through a window onto the ions of interest, ions which are trapped within the ICR (ion cyclotron resonance) cell of the mass spectrometer.

The wavelength-dependent IRMPD experiment allows the measurement of vibrational spectra of the gas-phase ion species, including putative reaction intermediates. This emerging infrared spectroscopic technique, combined with quantum chemical computations, is being used as an unprecedented tool for accurately characterizing the structures of gas-phase ions,[22] especially those of elusive species transferred from a reaction mixture.[9]

IRMPD spectroscopy adds a new dimension to MS, thus dramatically increasing the selectivity of this technique, even towards isomeric species. As recently reviewed,[9] this technique can be extremely insightful for mechanistic investigations, especially when several organometallic isomers can be hypothesized for a certain reaction pathway, or when intramolecular rearrangements occur. In this respect the group of Roithová is a leader in this specific field, and recently published an IRMPD study on a ruthenium-catalyzed C–C coupling between phenylacetylene and pyridine, thus reporting the characterization of several intermediate complexes.[23]

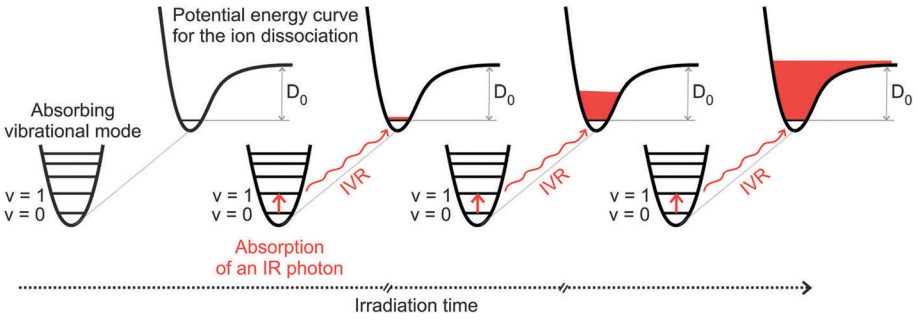


Figure 2. Infrared multiphoton dissociation spectroscopy: Sequential excitation-relaxation cycles (IVR = internal vibrational redistribution).[9]

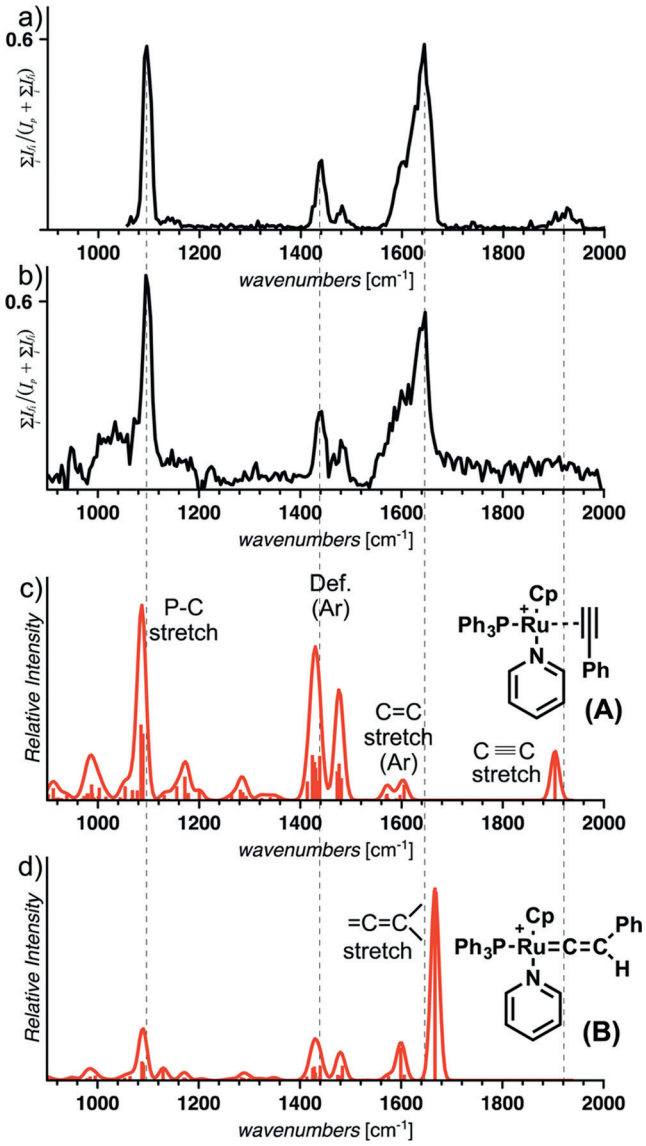


Figure 3. IRMPD spectra of the mass-selected complexes $[RuCp(PPh_3)(Py)(PhCCH)]^+$ from a reaction mixture when using harder (a) and softer (b) ionization conditions. Theoretical spectra of A (c) and B (d) isomers of $[RuCp(PPh_3)(Py)(PhCCH)]^+$.[23]

According to their results, when the triple bond of phenylacetylene is coordinated by ruthenium, isomerization occurs to form a vinylidene isomer. The coexistence of the two isobaric intermediates (i. e., the elusive π adduct and the vinylidene product) was inferred because the IR spectrum of the corresponding ion $[RuCp(PPh_3)(Py)(PhCCH)]^+$ shows both the diagnostic =C=C and C≡C stretching bands (Figure 3). In general, it can be observed that the combination of CID experiments and IRMPD spectroscopy allows complete characterization of the ions detected by ESI-MS. This combination allows one to check whether a certain species, resulting also from theoretical calculations, represents a possible intermediate along the reaction pathway. Indeed, it is worth noting that facilities which combine FEL infared sources and FTICR mass spectrometers are not easily accessible. As per our own experience, however, both European facilities accept projects from other research groups, and the results might be absolutely worth the effort.

2.4. Determination of the Intrinsic Enantioselectivity

During the last decade the group of Pfaltz, taking advantage of the pioneering works of the groups of Horeau,[24] Finn,[25] Chen and Hinderling,[1,26,27] developed an ESI-MS method for screening the performance of chiral catalysts towards a certain enantioselective reaction.[28] By means of mass-labelled quasienantiomeric substrates, it is possible to determine the enantiomeric ratios of catalytic intermediates, if the corresponding signals are visible in the ESI-MS spectrum (Figure 4[31b]). Pfaltz and co-workers have shown that if the targeted intermediate forms immediately before the enantioselectivity-determining step, the ESI-MS peak ratio of these quasienantiomeric transient species can predict the enantiomeric excess achieved in the bulk reaction. Accordingly, information about the intrinsic enantioselectivity of a catalyst could be obtained.[29] Alternatively, while investigating the mechanism of a certain reaction, a mass-labelled quasienantiomeric reagent can be employed to distinguish chiral intermediates by ESI-MS. If the relative abundance of these intermediates matches the experimental enantiomeric excess of the chiral product, it indicates that the species detected by ESI-MS are real intermediates involved in the enantioselectivy-determining step. Such a conclusion was even strengthened by the extension of Pfaltz's screening method for the determination of enantioselectivity by monitoring the back reaction, instead of the forward reaction, by

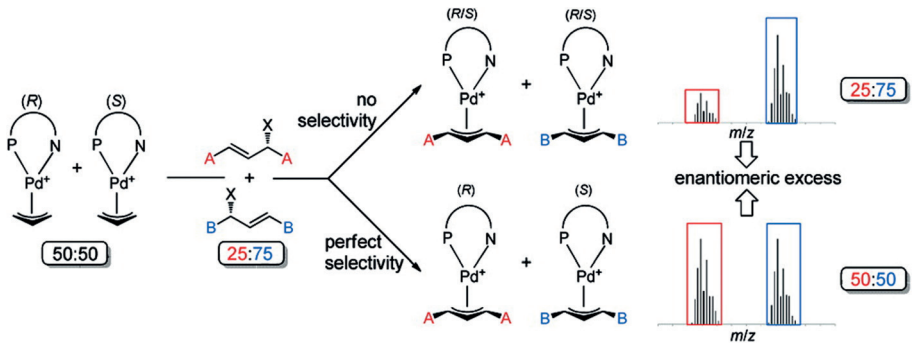


Figure 4. Schematic representation of the Pfaltz's MS method for screening the enantioselectivity of chiral catalysts.[31b]

ESI-MS.[30] In fact, according to the principle of microscopic reversibility, the forward and back reaction share the same transition state. Thus, it is possible to treat the quasienantiomeric final products with the same chiral catalyst used to prepare them, and detect the reaction intermediates of the back reaction. The high sensitivity of ESI-MS allows detection of the intermediates in the back reaction, even if they are in low concentration. It has been shown that the ratio of the ESI-MS signal intensities, for the intermediates, obtained from the back reaction reflects the enantioselectivity of the catalyst as well. The Pfaltz method has been successfully applied to allylic substitutions,[31] metal-catalyzed and organocatalytic Diels–Alder reactions,[32] and to Michael addition reactions.[33] More investigations, however, are needed to demonstrate how widely applicable the method is.

3. Mapping Routes of Multicomponent Reactions

In this section we review the most recent ESI-MS mechanistic studies of multicomponent reactions (MCRs). For the sake of completeness, organocatalyzed reactions, which share several similarities with MCRs, will also be covered. MCRs consist of one-pot reactions involving at least three substrates to assemble complex scaffolds, which usually have potential biological activity, in an atom-economical fashion. MCRs do not usually require metal catalysis even if some of them are mediated by transition metals. Organocatalyzed reactions also involve three or more organic substrates, but in this case one of them plays a catalytic role and will not be integrated into the final product.

The success of using ESI-MS to study the mechanism of such reactions rests on two main factors. First, the reagents involved in the aforementioned transformation, such as amines, carboxylic acids, phenols, isocyanides, and carbonyl compounds, are usually polar and thus susceptible to ESI ionization. Also the expected intermediates, in general, are ionic in nature (e.g., the nitrilium ion of the Ugi reaction) and thus demonstrate high ionization efficiency.[34] Moreover the unique ESI-MS selectivity, as well as the possibility offered by the technique to explore wide concentration arrays, enable mechanistic studies on complex multistep reactions, such as MCRs and organocatalyzed reactions. With the very high dynamic range of the more recent MS instrumentation, it is in fact possible to analyze both the reagents and reaction products at the same time as they are generally in high concentration. Intermediates, which would be in lower concentration, may also be detected.

Eberlin and co-workers pioneered the application of ESI-MS in 2004 by investigating the Morita–Baylis–Hillman (MBH) reaction: a multistep reaction, catalyzed by 1,4-diazabicyclo[2,2,2]octane (DABCO), which features a rather complicated catalytic cycle.[35] Besides this work, wherein crucial intermediates of the MBH reaction were intercepted and characterized for the first time, several other ESI-MS investigations have addressed a number of still unresolved issues regarding the mechanism of the MBH transformation. Recently, Neto and co-workers published a report on the MBH reaction, and they described the use of an imidazolium-based charged acrylate derivative as a reactant.[36] This approach, which was aimed at improving ESI-MS detection of the intermediates, allowed characterization of transient zwitterionic species and noncovalent adducts. In particular they identified thiourea-containing derivatives which are responsible for the enhanced reaction rate observed when using thiourea (Figure 5). The two hydrogen-bonding interactions of thiourea with the acrylate derivative are responsible for the noncovalent organocatalysis.

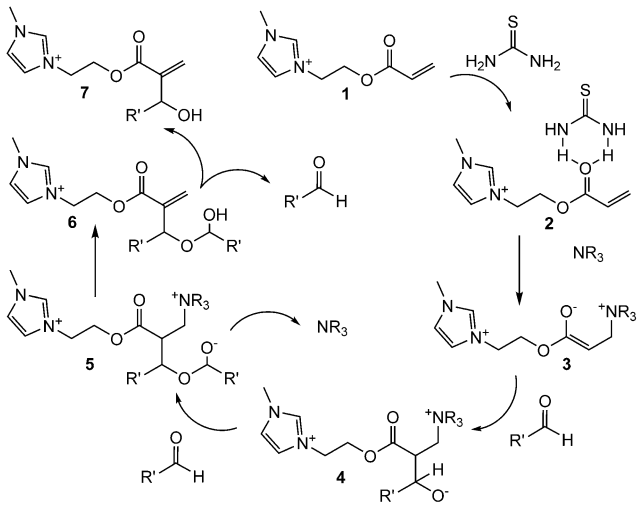


Figure 5. Reaction scheme for the Morita–Baylis–Hillman reaction. The intermediates **2**, **3**, **4**, and **5** have been identified by ESI-MS.[36]

The most intriguing mechanistic aspect of a MCR is the possibility of outlining several plausible reaction pathways, that is, different sequences of reagent combinations which would generate the final product in a convergent fashion. The recent comprehensive report on using ESI-MS to monitor the Hantzsch 1,4-dihydropyridine reaction is a great example of such complexity (Figure 6).[37] This MCR, which consists of the coupling of ammonia, an aldehyde, and two equivalents of a 1,3-dicarbonyl compound to form 1,4-dihydro-2,3,5,6-substituted pyridines, has six different plausible reaction routes.

Eberlin, Garden, and co-workers placed a quaternary ammonium ion tag at the periphery of either, or both, of the two key reactants, and monitored the composition of the reaction mixture over time. Even if the Hantzsch reaction intermediates are adequately polar, their low abundance in solution, combined with the matrix ion suppression effect, could prevent their detection by ESI-MS. In this study, the charge-tagging strategy minimizes ion suppression, thus allowing detection of a number of elusive reaction intermediates along a number of the proposed reaction pathways. Thus, they shed on light the many reaction pathways and demonstrate that multiple paths are possible, without affecting the regiochemistry and selectivity of the Hantzsch pyridine synthesis.

Incidentally, very recently, Cooks and co-workers investigated the ability of ESI to accelerate the Hantzsch reaction.[38] By analyzing the reaction mixture composition

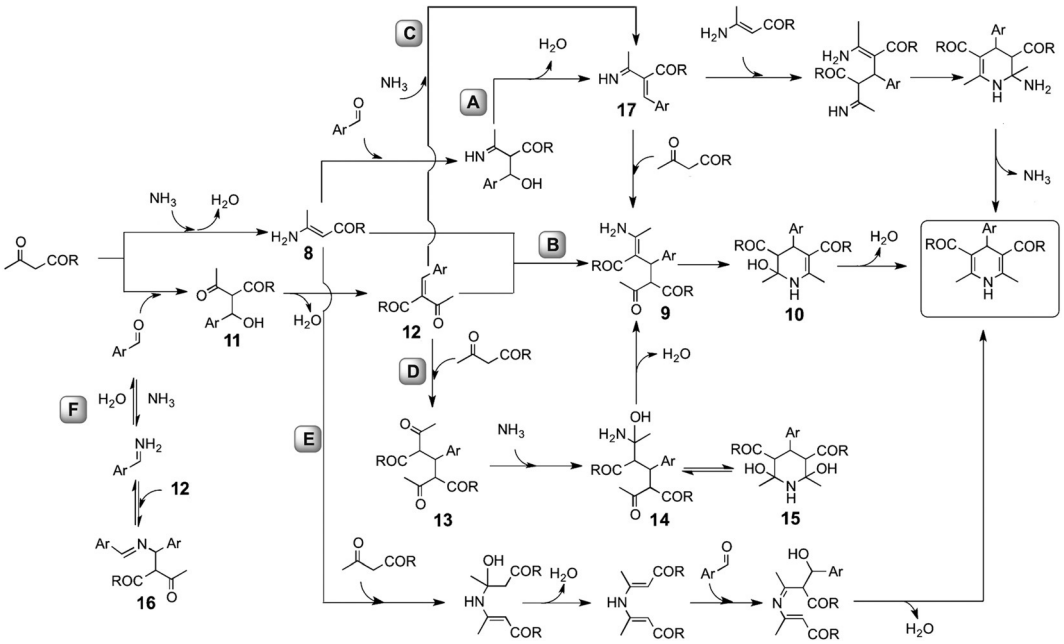


Figure 6. Possible mechanistic paths (A–F) for the Hantzsch reaction. The intermediates **21**, **9**, **10**, **11**, **12**, **13**, **7**, **8**, **5**, and **14** have been identified by ESI-MS. Data suggest the predominance of three convergent paths: B, C, and D.[37]

by ESI-MS they detected several of the expected intermediates. In particular, in offline nano-electrospray experiments with UV and NMR quantification they demonstrated that the reaction rate is improved in the spray droplets.

Indeed, it is known that the rate of some reactions can be enhanced when they are forced to occur in the confined small volume of the electrosprayed charged droplet.[4] It is worth nothing that, in such cases, MS can be moved from the usual analytic dimension to a preparative one, and that the products synthesized in this way can be collected and characterized separately.

This ESI-MS feature takes advantage of electrospray tuning, which promotes the encounter between reactants present in the electrosprayed solution.[4,38] Moreover, the electrospray microdroplet might serve as a confined space where acid-catalyzed reactions can occur more easily.

Back to the identification of reaction intermediates, in addition to the Hantzsch and Morita–Baylis–Hillman reaction several research groups over the last few years have extensively explored the domain of organic transformations proceeding via iminum or enamine intermediates. Roithová and co-workers focused on the organocatalytic cascade reaction between an α,β-unsaturated aldehyde and a malonate derivative, for the enantioselective preparation of cyclopentane rings.[39] The employment of a chiral secondary amine as an organocatalyst is also advantageous for ESI-MS monitoring of the reaction mixture. Iminium adducts, in fact, formed by condensation of the amine with the reactants first and finally with the product, can be sampled efficiently by ESI-MS, thus allowing the characterization of the various reaction intermediates. Most interestingly tandem MS experiments opened a window to the crucial cyclization event. Only a very small amount of the protonated intermediate, which undergoes ring closure, forms before the intramolecular step and can thus be isolated in the gas phase and activated by CID for its characterization. This evidence corroborates the hypothesis that the cyclization event is a prerogative of suitable neutral enamine intermediates.[39]

In a study on the synthesis of pyrazolones from Morita–Baylis–Hillman adducts by Coelho and co-workers,[40] hydrazinium ion intermediates were intercepted by ESI-MS, and the overall reaction mechanism of pyrazolone formation was investigated by employing ESI-MS/MS.

By employing an innovative approach, a transient aliphatic iminium ion has been recorded for the high-temperature Eschweiler–Clarke secondary amine methylation.[41] The peculiar high-temperature reaction conditions of this last transformation, in fact, have been reproduced by employing a PATI (paper assisted thermal ionization) source for the mass spectrometric reaction mechanism investigation. This ambient ionization technique is indeed a useful MS configuration for mechanistic studies. The reaction mixture is loaded onto a heated metal probe which works as a reaction vessel. A triangular piece of filter paper helps ionization of the hot reactant solution. PATI was also employed for the detection of intermediates for reactions occurring at room temperature, such as dioxygenation of a number of alkenes.[41]

In an outstanding contribution Wennemers, Pfaltz, and co-workers challenged the organocatalytic conjugate addition of aldehydes to nitroolefins by ESI-MS.[33b] For this purpose they employed a series of H-D-Pro-Pro-Xaa tripeptides, which are well-known and efficient chiral organocatalysts for such addition reactions. They first detected and characterized putative enamine intermediates by ESI-MS, and then proposed a reaction mechanism after ruling out a plausible enol mechanism. Mass-labelled quasienantiomers of an α-substituted aldehyde were prepared and treated with the tripeptide chiral catalyst. According to the microreversibility principle,

the back reaction occurs and they were able to detect the same transient species as seen for the forward addition reaction, by ESI-MS. The ESI-MS relative abundance of quasienantiomeric enamines formed in the back reaction mismatched the enantiomeric excess observed for the forward reaction. This data strongly suggests that the C–C bond-forming step is not the enantioselectivity-determining step for this reaction, thus indicating that the last protonation, after addition of the enamine to the olefin, is the turnover-limiting step. Such speculation is consistent with the observed rate acceleration by addition of an acid, and also affects the reaction enantioselectivity.[33b]

Currently, an in situ formed iminium ion is also accepted as the first intermediate in the mechanism of the Ugi multicomponent reaction. This reaction, in its original formulation, converts an aldehyde, an amine, an isocyanide, and a carboxylic acid into peptidomimetic products. The mechanism of this four-component reaction (U-4CR) is under debate because two different convergent pathways have been postulated, starting from the mentioned in situ formed Schiff base (Figure 7). Two mass spectrometric investigations have

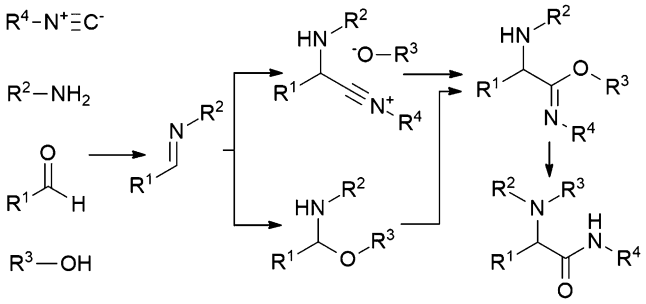


Figure 7. Possible mechanistic pathways for the Ugi and Ugi–Smiles multicomponent reactions (R^3 = acyl for the Ugi reaction, R^3 = aromatic residue for the Ugi–Smiles reaction).[2d]

addressed this issue, one from De Angelis and co-workers,[2d] and the other by Eberlin and co-workers.[2f] They both report, unambiguously, the characterization of a nitrilium ion intermediate (Figure 8), which results from isocyanide trapping of the iminium ion. De Angelis and co-workers employed conventional neutral reactants[2d] while Eberlin and co-workers used an imidazolium-based charge-tagged amine.[2f] The elusive nitrilium ion species is specific for only one of the two alternative hypothesized reaction routes (Figure 7). In the alternative reaction path the imine reacts with the carboxylic acid, thus leading to a neutral hemiaminal intermediate. The hemiaminal was not detected, either by employing the charge-tagging strategy or by exploiting the reaction conditions to force its formation.[2d,f] For this reason the second proposed reaction path was rejected, in accordance with theoretical calculations.[42] Similarly, the Smiles variant of the Ugi reaction was addressed by ESI-MS, thus showing that a nitrilium ion mechanism is also operational.[2d] In the study of the charge-tag devoid U-4CR the use of an ionic hook is questioned. In such a case, in fact, the intermediate/transition-state-forming step of the reference reaction might be altered

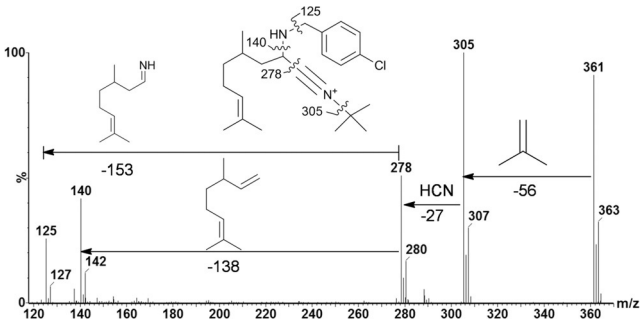


Figure 8. ESI(+)-MS/MS of nitrilium ion intermediate at $m/z = 361–363$.[2d]

because of charge-biased thermodynamics and reaction kinetics.

Organocatalysis has also emerged as a powerful tool for efficient metal-free enantioselective syntheses, and therefore the interest in the role of the organic chiral inducer in the catalytic cycle has grown enormously. Recently Engeser and co-workers synthesized a charge-tagged 4-hydroxy-L-proline derivative with the aim of studying the inverse asymmetric aldol reaction between aldehydes and diethyl ketomalonate by ESI-MS.[43] The significantly enhanced ESI sampling ability of the charged L-proline adducts, formed along the reaction pathway, ensured their convenient monitoring without affecting the overall reactivity of the catalytic system. Such an approach allowed MS characterization of the intermediates, and they were in accordance with the enamine-based List–Houk mechanism for this reaction.[43]

In the context of organocatalysis, the progress of a thiazolylidene-catalyzed benzoin condensation reaction was recently monitored by ESI-MS by exploiting a thiazolium derivative carrying a sulfonate charge tag. Intermediates in the benzoin condensation were fished out of the reaction mixture and characterized by MS/MS, and theoretical calculations supported the structural assignments. These data show the viability of negatively charged compounds, which both catalyze and assist in tracking reactions involving N-heterocyclic carbene catalysts.[44]

With the aim of elucidating catalytic reaction pathways, ESI-MS has been successfully employed to identify the catalytic role of phosphines in organic transformations. In situ generated organophosphonium intermediates are proposed to be crucial for phosphine-based organocatalysis. Indeed such charged species are perfectly suitable for being fished out and studied by ESI-MS. In 2014 McIndoe and co-workers investigated the conjugate addition of an alcohol to a butynoate ester using an organophosphine catalyst.[45] A notable online monitoring of the reaction mixture was achieved by using their PSI-ESI-MS method.[15] It shows the dynamic formation of several organophosphonium intermediates, including transient species which then lead to oligomeric by-products. The interest in such organophosphonium species is not limited to this peculiar reaction, but it might extend to several related transformations which are thought to proceed by similar paths (cycloaddition reactions, conjugate- and hetero-additions, MBH, etc.).[45]

4. Homogeneous Metal Catalysis

ESI-MS can be regarded as the ideal tool to study the mechanism of homogeneous metal-catalyzed reactions. Its capability of tracking multiple metal complexes present in trace amounts in complex solutions, without perturbing their coordination sphere, places this technology in a prominent position within the pool of spectroscopic techniques.[6] The requirement for the species of interest to be charged is not a limitation for the ESI-MS study of metal complexes, because they are either already charged or ionized by protonation during the spray generation. There are also cases where the cationic and anionic portions of the complex dissociate in the gas phase, thus allowing inspection of the positively charged metal-ion moiety. In some cases the introduction of a charged moiety on one of the ligands makes it possible to study zero-valent metal complexes. Thus, in principle, all metal-ion complexes are suitable objects for ESI-MS investigation. In this section we review the latest ESI-MS investigations of organometallic reactions in solution.

It is not surprising that many recent reports in the field deal with palladium-mediated reactions. Palladium is one of the most convenient metals used in catalytic reactions, but it is also expensive. The amount of palladium used for a reaction can be reduced by several orders of magnitude upon optimization of the reaction procedure. In addition, the determination of the best palladium catalyst for a certain transformation is an expensive and time-consuming activity. However, in principle, selection of the ideal palladium catalyst, as well as the amount needed, for a specific transformation can be more efficient if detailed knowledge of the mechanistic pathway is available.

The Negishi palladium-mediated cross-coupling reaction, studied for the first time by ESI MS in 2010,[46a] has also been recently investigated by Cooks and co-workers, who track the reaction by using an inductive ESI-MS-based online monitoring approach.[13a] This technique, which differs from McIndoe's PSI-ESI-MS approach, is based on pulsed voltages to generate the electrospray (see Section 2), allowing to monitor the composition of the air-sensitive Negishi reaction mixture (Figure 9).

The unprecedented observation of organozinc adducts challenged the classic view of the Negishi reaction, thus suggesting that the zinc reagent may not be released from the complexes throughout the palladium catalytic cycle (Figure 10). Such an observation has been recently corroborated by the ESI-MS study of Koszinowski and co-workers[46b] who employed a combination of different instrumental techniques.

Also interesting is that the inductive ESI-MS-based system was effective for monitoring heterogeneous reactions. The time-dependent occurrence of three different intermediates (a short-lived one with a life-time of ca. 5 s), in the Pd/C-catalyzed hydrogenation of an aldehyde, was revealed by collecting selected ion chronograms and time-resolved mass spectra.[13a]

The relatively easy sampling by ESI-MS of palladium complexes has also been exploited for the investigation of the palladium-mediated desulfitative synthesis of aryl ketones.[46] In this recent contribution, the reaction was investigated by

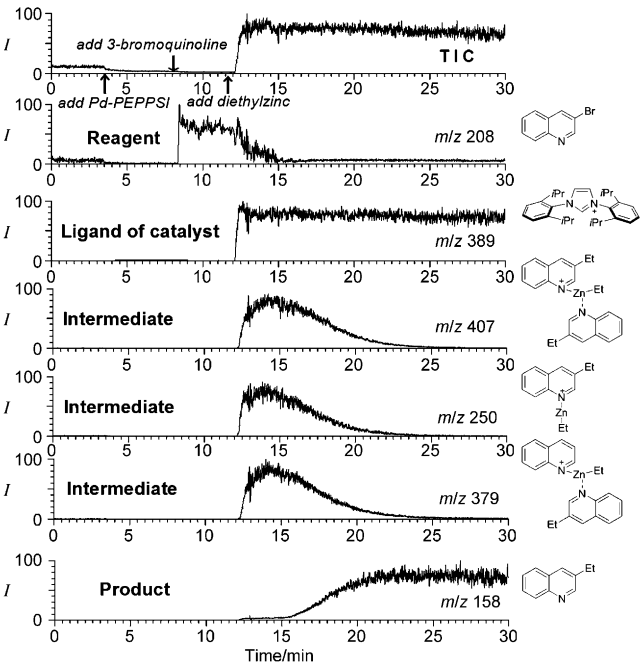


Figure 9. Total ion chronogram and selective ion chronograms of 3-bromoquinoline (*m/z* 208), Pd ligand (*m/z* 389), intermediates (*m/z* 407, 250, 379), and the product ethylquinoline (*m/z* 158) in the Negishi cross coupling of 3-bromoquinoline with diethylzinc. *I* = relative abundance.[13a]

using acetonitrile and a sodium arylsulfinate as starting materials. The analysis of the reaction mixture resulted in the observation and characterization of the cationic portion of five palladium-containing intermediates. This finding confirmed the formation of the Pd–aryl[47] bond by loss of SO_2, which was replaced by CH_3CN, in the palladium coordination sphere. The next step was the crucial carbopalladation of acetonitrile.

Remarkably this catalytic step, which formally is an isomerization, is revealed by the presence of product ions, specific to the two isomers, in the tandem mass spectra.[46] Similarly in a recent study of the Heck arylation of allylated malonates, the C–C bond-forming reaction during aryl insertion into the terminal carbon atom of a palladium-coordinated allylated malonate was observed by ESI-MS.[48] Interestingly, in this investigation, which is based on detection of palladium(II) intermediates by ESI-MS, a possible chelation of palladium by both carbonyl groups of the reactant was also suggested. Scheme 1 shows some of the more significant palladium intermediates, as discussed above, which were detected and characterized by ESI-MS.

Determining palladium coordination can be crucial, especially when enantioselective syntheses are concerned. In particular mass-labelled quasienantiomers of allylated malonate have been employed to screen the intrinsic enantioselectivity of chiral palladium catalysts.[31] Under specific reaction conditions, traces of these substrates eliminate acetyl acetonate and lead to the formation of mass-labelled quasienantiomeric intermediates. Such palladium(II) intermediates, which are easily detected by ESI-MS, are the same as those occurring during palladium-catalyzed allylic substitution.

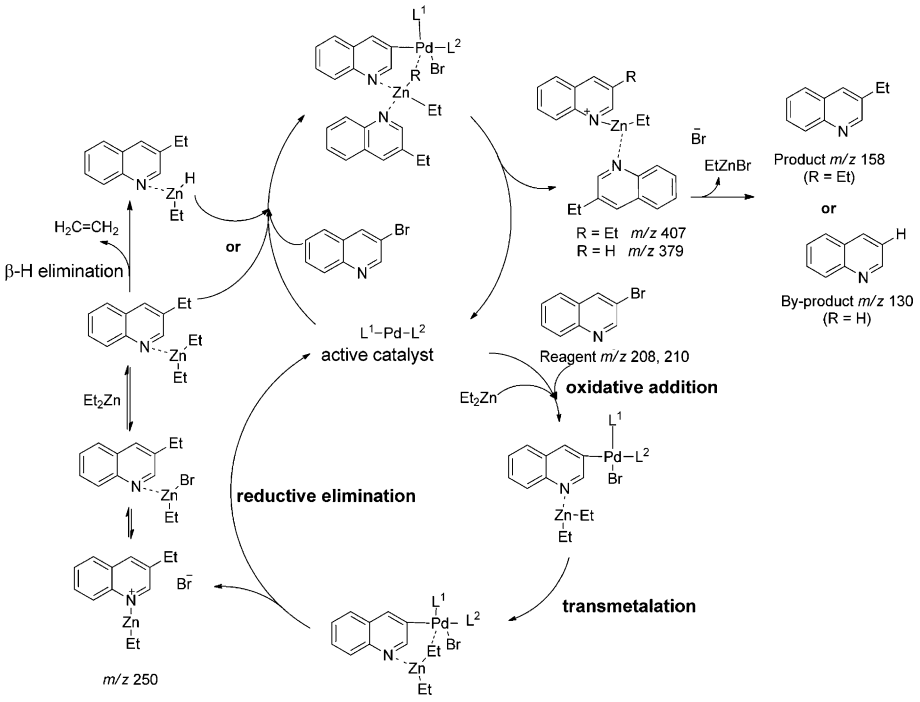
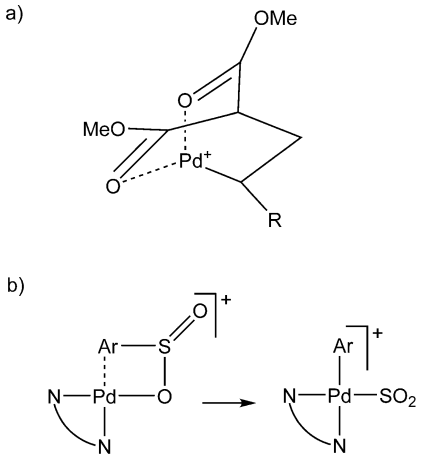


Figure 10. Proposed mechanism of the palladium-catalyzed Negishi cross-coupling of 3-bromoquinoline with diethylzinc.[13a]



Scheme 1. Significant palladium intermediates identified by ESI-MS. a) From Ref. [48]; b) from Ref. [46c].

Very interestingly, their relative abundance in the mass spectra has been demonstrated to match the enantiomeric excess of the allylic substitution product. The chirality-dependent formation of such transient species is in accordance with the microreversibility principle. For the case of palladium-catalyzed allylic substitution it was shown that palladium-based intermediates present in solution can be observed by ESI-MS.[28–30]

Neto and co-workers have also addressed palladium catalysis by looking at the mechanism of the phosphine-free neutral Heck reaction.[49] To track the mechanism they synthesized a charged imidazolium-based ligand to get an overall charge-tagged palladium catalyst. This complex was employed to detect a number of intermediates for the reaction between styrene and 4-iodoanisole.

Rhodium-mediated transformations constitute another group of highly intriguing reactions. McIndoe, Weller, and co-workers very recently reported on the investigation of two reactions in this field by using PSI-ESI-MS.[16,50] They investigated the hydroboration of *tert*-butylethene (TBE) with amine-boranes, catalyzed by $[Rh(xantphos)]^+$, by using ESI-MS online monitoring.[50] Several rhodium-containing intermediates responsible for B–H activation through the metal were intercepted at the early stage of the reaction progress. Interestingly, their signals decrease very quickly over time, and thus explain why they cannot be detected by NMR spectroscopy. PSI-ESI-MS, in agreement with other experimental data, indicates release of the hydroboration product as the rate-determining step of the reaction. This year they published a paper dealing with the observation of the mechanism of the selective rhodium-mediated partial hydrogenation of alkynes by using PSI-ESI-MS.[16] The charge-tagging approach was exploited by employing an unprecedented combination of substoichiometric amounts of a charged phosphine ligand and a charged unsaturated substrate. This combination allows tracking of the rhodium adducts over time, as well as substrates and reaction products (Figure 11). The high point density resulting from the PSI-ESI-MS online monitoring allowed high-quality kinetic data to be obtained. Also the dependence of such data on the temperature of the reaction mixture does confirm that the reaction occurs in solution and is not affected by the ESI process.[16]

ESI-MS recently yielded remarkable results for exploration of nickel-mediated transformations. Scheley and Fu investigated the mechanism of enantioselective nickel-cata-

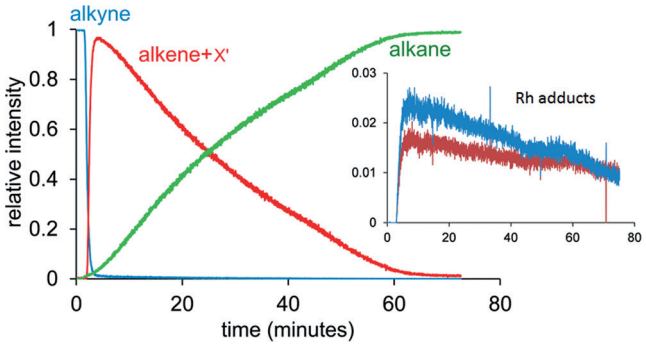


Figure 11. PSI-ESI-MS chronogram for hydrogenation of the tagged alkyne under 3 psi of H_2. Inset: Relative intensity versus time plot exhibiting the behavior of Rh adducts.[16]

lyzed Negishi arylation of propargylic bromides using a wide range of tools, including ^{19}F NMR (on a F-substituted aryl derivative) and EPR spectroscopy, and X-ray methods.[51] They convincingly envisioned a radical-chain mechanism in which nickel passes through the nickel(I), nickel(II), and nickel(III) oxidation states. They characterized the predominant aryl/nickel resting state of the catalyst by ESI-MS, and also obtained consistent confirmation by using other spectroscopic analyses.

In another contribution, the mechanism of an unprecedented nickel-catalyzed hydroselenation of terminal alkynes was studied by offline ESI-MS.[52] The analysis of the methanol reaction mixture showed several putative nickel-containing intermediates as protonated ions. In particular, one of them was found to undergo protodemetallation during the CID gas-phase experiment, thus affording the final diene product. This diene was claimed as evidence of the reactivity of the intermediate reactivity.

Additionally, Eremin and Ananikov studied $[Ni(acac)_2]$ to investigate the eventual formation in solution of Ni_xO_y superatomic species.[53] This species is a combination of several atoms forming a particular core structure with new electronic and chemical properties. It can be considered a self-standing assembly, which shows interesting chemical reactivity in its use in organometallic chemistry. Among the various nickel clusters, sampled by ESI-MS, from the above-mentioned solution, the binuclear $[Ni_2(acac)_3]^+$ was identified as the only ion containing the superatomic Ni_2O_2 core. Tandem MS experiments on this ion demontrated the exceptional reactivity of Ni_2O_2, which is capable of cleaving C–C, C–H, and C–O bonds.[53]

Among the transition metals, iron has attracted much attention because of its possible employment as a sustainable substitute for other expensive metals in redox catalysis. Iron-containing intermediates along the pathway of redox reactions can be readily studied by ESI-MS without any restriction as far as their oxidation state is concerned.[6] Che and co-workers reported the ESI-MS identification of iron(III) and iron(IV) species as intermediates of the oxidation reaction of alkanes mediated by nonheme iron complexes.[22g] In particular the iron(IV) complex containing the Fe=O moiety was considered an effective catalyst form. Another cationic complex, which includes an iron(IV) ion has been intercepted as a putative intermediate of the nitrene/imide insertion and transfer reaction using arylamines.[54]

In a recent contribution from our group, we investigated the copper-catalyzed azide alkyne cycloaddition (CuAAC) by offline ESI-MS(/MS).[2e] Its challenging mechanism had been previously addressed by several theoretical and experimental studies, which suggested a mechanism involving binuclear copper intermediates. We then analyzed two different CuAAC reaction mixtures, one containing classic neutral reactants and the second containing imidazolium-based charged alkyne. The approach, in both cases, allowed, for the first time, interception and full characterization of the crucial bis(copper) intermediates like bis(copper) acetylides and triazoles (Figure 12). It is worth nothing that the use of

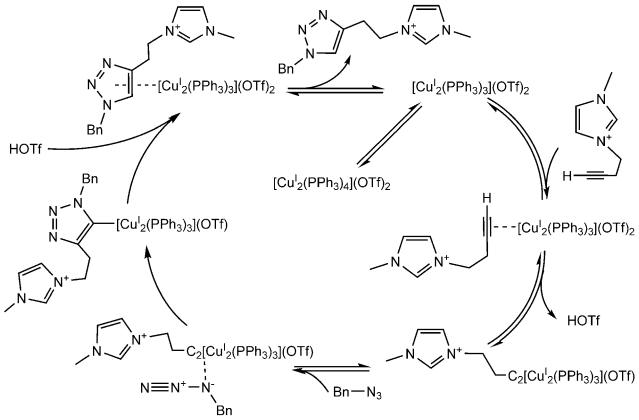


Figure 12. Catalytic cycle for the CuAAC reaction carried out by using the imidazolium-based charged alkyne.[2e] The bis(copper) intermediates reported in the proposed catalytic cycle have been identified and characterized by ESI-MS/MS.

neutral reactants and ion-tagged substrates results in complementary information for the identification of the intermediate. Recently, bis(copper) acetylide and bis(copper) triazole intermediates of the CuAAC reaction were isolated in the condensed phase and characterized, and are proposed as intermediates for the CuAAC catalytic cycle.[55]

Homogeneous ruthenium and gold catalysis have also been recently investigated. Roithová and co-workers challenged the mechanism of ruthenium-catalyzed C–C coupling between phenylacetylene and pyridine.[23] In particular they characterized an unprecedented crucial intermediate, wherein the alkyne is coordinated by the metal cation through a π bond. The energy of this interaction was found to be significantly high by energy-resolved tandem MS and gas-phase ion-molecule reaction experiments. Additionally all the intercepted intermediates have been unambiguously characterized by IRMPD spectroscopy, thus matching the mechanistic hypothesis proposed for this reaction.

The occurrence of elusive gold(I) α-oxo carbenes, thought to be intermediates in the oxidation of alkynes, have also been investigated by Roithová and co-workers.[17] They analyzed a dichloromethane reaction mixture containing an alkyne, pyridine *N*-oxide, and a gold(I) catalyst, and they detected several gold(I) intermediates. The initial formation of β-

gold(I) vinyloxypyridinium was evidenced, and it subsequently rearranges into either an α-oxo carbenenoid (a synthetic surrogate of the α-oxo carbenes) or to a gold(I)/pyridine adduct. The occurrence of rearrangements of the gold complexes in solution was monitored by time-dependent CID tandem MS experiments. The structure of isobaric ions was assigned by IRMPD spectroscopy and DFT calculations. Moreover the actual existence in the reaction mixture of the intercepted intermediates is corroborated by isotope-labelling experiments. Taken together, all the above-mentioned data allow one to definitely rule out all possible ESI artifacts.

In our opinion, this comprehensive MS-based study by Roithová and co-workers,[17] together with the outstanding online monitoring exercises from the groups of McIndoe[16] and Cooks,[13a] can be considered the most impressive and reliable contribution to mechanistic investigations by using ESI-MS.

5. Monitoring Polymer Growth

More than 15 years ago, the pioneering studies of the polymerization of alkenes by ESI-MS paved the way for prolific research focused on mechanistic investigations of organic reactions by using this technique. The ground-breaking paper of Chen and co-workers on ring-opening metathesis polymerization (ROMP)[1,56] also introduced the original idea of the charge-tagging strategy for effective ESI sampling of neutral intermediates.[26] Later on Santos and Metzger, who studied the Ziegler–Natta and Brookhart polymerization, introduced the continuous online monitoring approach which significantly improved ESI-MS capability and led to insight into the polymerization mechanisms.[57]

Nowadays, the interest in ESI-MS applications in the polymer field has been boosted by new online monitoring techniques, and by the opportunity to address the intriguing processes of nanoparticle formation and noncarbon-based construction of polymers. One of the most recent examples demonstrating the potential of using ESI-MS to investigate polymerization mechanisms was been published by Machado and co-workers.[58] In their contribution on styrene polymerization catalyzed by iron-containing ionic liquids, ESI sampling and MS characterization of styrene chloronium ions was achieved using BMI·Fe_2Cl_7 as the catalyst (BMI = 1-*n*-butyl-3-methyl imidazolium). These species were identified as key intermediates responsible for polymerization initiation. Moreover the BMI-containing chloronium cation clusters confirmed the anticipated positive effect of the ionic liquid in the catalytic event resulting from the chloronium intermediate stabilization.[58]

Junkers and co-workers reported a new flexible setup which consists of an online microreactor/ESI-MS coupling, which allows real-time monitoring of a polymerization reaction.[59] This apparatus was employed to monitor a reversible addition-fragmentation chain-transfer (RAFT) polymerization. Moreover, this method was also effective for single unit monomer insertion (SUMI), thus resulting in useful information for quick optimization of flow reaction conditions.[59]

The potential of ESI-MS for monitoring reactions also expanded to the area of inorganic reaction mechanisms. Particularly, in 2014, the intriguing formation process of thiolated gold nanoclusters (NCs) was addressed by ESI-MS.[60] The fascinating properties of such NCs might be related to their growth mechanism. In the ESI-MS time-resolved study by Jiang, Xie, and co-workers, the gold(I) precursor and the gold NC intermediate species were identified and monitored over time (Figure 13). This time course of gold NC formation revealed the existence of an initial kinetically controlled reduction-growth mechanism and subsequent thermodynamically guided intercluster conversion into $[Au_{25}(SR)_{18}]^-$.[60]

In the growing research area of B–N (boron-nitrogen) materials, such as those isoelectronic with polyolefins and white graphene, Weller, Macgregor and co-workers recently reported a mechanistic investigation of the iridium-mediated dehydropolymerization of amine-boranes.[61] Moving from sterically hindered amines to ammonia, they characterized iridium-containing intermediates by ESI-MS. In particular the dehydrogenation adduct of $H_3B{\cdot}NMe_2H$ was intercepted, as a first dehydrocoupling product of $H_3B{\cdot}NMeH_2$, and multiple iridium-bound oligomers for $BH_3{\cdot}NH_3$. This experimental evidence corroborates the unfavorable B–N coupling tendency induced by the steric bulk of the amine, as anticipated by theoretical calculations.

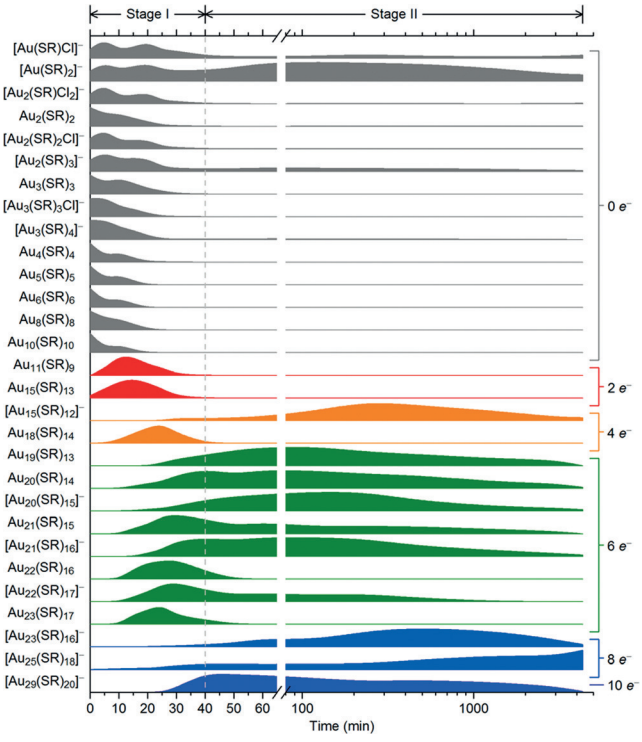


Figure 13. Normalized ESI-MS spectral intensity profiles of the complex and NC species throughout the synthesis.[60]

6. Conclusions and Perspectives

The recent impressive advances of ESI-MS in terms of sensitivity and selectivity, as well as its integration with ancillary techniques, such as ambient ionization, MS/MS, and IRMPD, have firmly established its role for investigating reaction mechanisms in solution, thus complementing traditional tools. Among the many alternative ionization methods being used for mechanistic studies, desorption electrospray ionization coupled to MS (DESI-MS) has started to play an important role.[4] Although beyond the scope of this Minireview, we cite a few examples. An asymmetric palladium-catalyzed conjugate addition reaction of arylboronic acids to enone substrates has been investigated mechanistically by DESI-MS, thus leading to the identification of intermediates along the catalytic cycle and allowing differentiation the substrate reactivity.[62] In polymer growth studies, DESI-MS was used for real-time monitoring of the homogeneously catalyzed polymerization of ethylene.[63] The reaction progress was monitored, and the catalytic reactive species were identified by tandem MS.

The implications of the progress of ESI-MS for investigating organic reaction mechanisms are truly exciting. Beyond the intrinsic charm of the approach, the development of new routes for increasing the effectiveness and efficiency of chemical processes can be realized based on reliable information on the reaction mechanism. Although ionizable intermediates are necessary for detection using MS, the possibility of using charge-tagged reagents has been extended to the detection of reaction intermediates and transient non-ionizable species. Moreover, the acceptance of ESI-MS as a way for monitoring reactions has greatly benefited from recent studies which give direct and indirect evidence of the correlation between putative reaction intermediates intercepted in the gas-phase and those existing in solution.

The outstanding achievements of MS in the last two decades, mainly resulting from the development of the soft ionization techniques, are notable. Research areas such as the chemistry of natural products, noncovalent interactions, drug–biological target interactions, as well as new fields such as proteomics and metabolomics, biochemistry, and biomedicine in general, have benefited notably from such developments. In the arena of reaction mechanisms, elucidation of the ESI-MS approach for exploring reactions in solution is now only in its inception. Beyond the skepticism of taking reaction intermediates from solution into the gas phase, we have tried to demonstrate the enormous potential of ESI-MS for intercepting transient short-lived and low-abundance intermediates, the structural characterization of which can definitely exploit CID and MS^n techniques, as well as IRMPD spectroscopy for obtaining experimental IR spectra for comparison with calculated ones. This comparison allows elucidation of different isomers, and even of protomers present both in solution and in the gas phase. An efficient real-time monitoring of all the species taking part in the overall reaction course is also possible. The possibility of following multicomponent reactions and one-product convergent multimechanism reactions, as well as, homogeneous metal-catalyzed and proton-catalyzed reactions is absolutely exiting.

Future challenges in the development of the ESI-MS strategy for studying reaction mechanisms depends on two things. For one, chemists should be made aware of the power of the technique to increase their confidence of this approach. Secondly, as far as instrumentation, it is envisioned that new developments of the technique in terms of sensitivity, computerized data treatment, and the possibility of carrying out reactions directly in the ESI plume will produce results for an even larger number of organic reactions. In some cases reaction mechanisms will be studied first time, and in other cases the study will be used to confirm a so-called "well consolidated" mechanism. Moreover, additional development and easy accessibility of the ancillary techniques, like ion mobility and IRMPD, for obtaining information on significant conformational structures of intermediates, accompanied by calculation of their energies, will again increase interest.

Finally, any improvement in the synthesis of important molecules stemming from ESI-MS mechanistic studies will represent a tremendous boost.

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