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Observations of Rhodium-Containing Reaction Intermediates using HPLC with ICP-MS and ESI-MS Detection

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Abstract: HPLC with ICP-MS or ESI-MS detection has been used to investigate metal-containing reaction intermediates in the ligand exchange process leading to formation of the well known $Rh_2(MEPY)_4$ catalyst. A variety of intermediates are observed along the pathway to formation of the desired tetrasubstituted product, including isomeric species from di-, tri-, or tetrasubstitution that were previously believed to be absent.

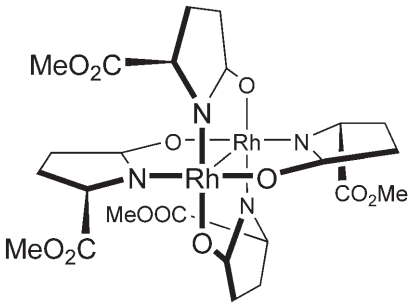
Keywords: catalyst; ESI-MS; HPLC; ICP-MS; metal speciation; rhodium

Metals play an increasingly important role in the synthesis of pharmaceuticals.[1] Consequently, detailed studies of metal-containing catalysts and/or intermediates, by-products and impurities are becoming increasingly important.[2] In addition to the conventional tools for organic analysis, metal-containing species can be characterized using ICP-MS,[3] ICP-AES,[4] X-ray fluorescence,[5] electrochemistry,[6] and atomic absorption spectroscopy.[7] Of these techniques, ICP-MS is perhaps most powerful, allowing highly sensitive quantitation of a wide variety of different metals.[8] A drawback of the technique, as typically applied, is that all metals of a certain type, e.g., Pd, are counted together, irrespective of oxidation state or the presence/absence of coordinating ligands. However, when coupled with HPLC or other separation techniques, ICP-MS can provide information on the metal content of chromatographically resolved species,[9] suggesting a possible role in the study of metal-containing species in organic reactions, catalyst degradation, or recalcitrant metal impurity problems.

While widely studied in environmental analysis,[10] metal speciation tools are seldom used in pharmaceutical process research. We therefore undertook an investigation into the utility of HPLC-ICP-MS, as well as more conventional HPLC-ESI-MS, in the study of a model ligand exchange reaction: the formation of dirhodium(II) tetrakis[methyl-2-oxopyrrolidin-5(*S*)-carboxylate], $Rh_2(MEPY)_4$, from rhodium acetate and methyl pyroglutamate.

The $Rh_2(MEPY)_4$ catalyst is one of a series of enantioselective catalysts developed by Doyle and co-workers[11] and is based on amide ligand substitution around a dirhodium core.[12] These catalysts have proven useful in a number of enantioselective transformations, especially enantioselective cyclopropanation and CH insertion processes involving the formation of transient metal-carbene intermediates.[13] The structure of the reported catalyst (Scheme 1), based on X-ray crystallographic evidence,[11] has two oxygens and two nitrogens bound to each rhodium, with the two nitrogens (or two oxygens) oriented *cis* to one another: the (*cis*-2,2) configuration.

The formation of dirhodium(II) carboxamidate compounds from rhodium acetate has been well studied[12–14] and is believed with the $Rh_2(MEPY)_4$ catalyst to involve the successive displacement of acetate ligands from the rhodium acetate precursor by methyl pyroglutamate (MEPY). Catalyst formation is complicated by



Scheme 1. (*cis*-2,2)-Rh_2(4*S*-MEPY)$_4$.

the potential presence of numerous isomeric species resulting from different geometries in the addition of enantiopure MEPY ligands to the dirhodium core. For example, five isomeric species are possible for the $Rh_2(OAc)_2(MEPY)_2$ intermediate, while the tri-MEPY and tetra-MEPY species can each exist in as many as four isomeric forms. Interestingly, despite the possibility of four different isomers for the $Rh_2(MEPY)_4$ product, the *cis*-2,2 isomer is the only species that has been reported to date.[11,12]

In the reaction leading to the formation of $Rh_2(MEPY)_4$ one could anticipate the presence of as many as 15 different rhodium-containing species. Thus, the MEPY catalyst formation system met several of our criteria for evaluation of metal speciation tools. The system possessed sufficient complexity to be of interest. The system was well known, having already been studied by conventional analytical techniques.[11] Nevertheless, several mysteries remained that merited further investigation. Ligand exchange was known to be sluggish in this system at room temperature, and the catalyst starting material and product were known to be fairly stable to exposure to oxygen and routine chromatographic eluents. Accordingly, we had a reasonable expectation of being able to chromatographically resolve the various species.

Initial feasibility analysis involved the preparation of $Rh_2(MEPY)_4$ on a 200-mg scale, using an established procedure,[14] with removal of aliquots over time. Aliquots were evaporated, then dissolved in acetonitrile and injected within a single chromatographic run, using the technique of flow injection analysis (FIA) HPLC-ESI-MS. In the resulting chromatogram (Figure 1) diagnostic masses for starting material, product, and each of the intermediates have been identified, and are expressed as separate channels for convenient assessment of relative abundance. The set-up of a method of this sort is rapid, and analysis of the collection of 30 reaction aliquots takes less than 15 min. Interestingly, the various intermediates in the formation of MEPY can be seen to change over the course of the reaction, giving some indication of reaction kinetics, and bringing to light unexpected findings. For example, literature precedent suggested that the third and fourth ligand substitutions

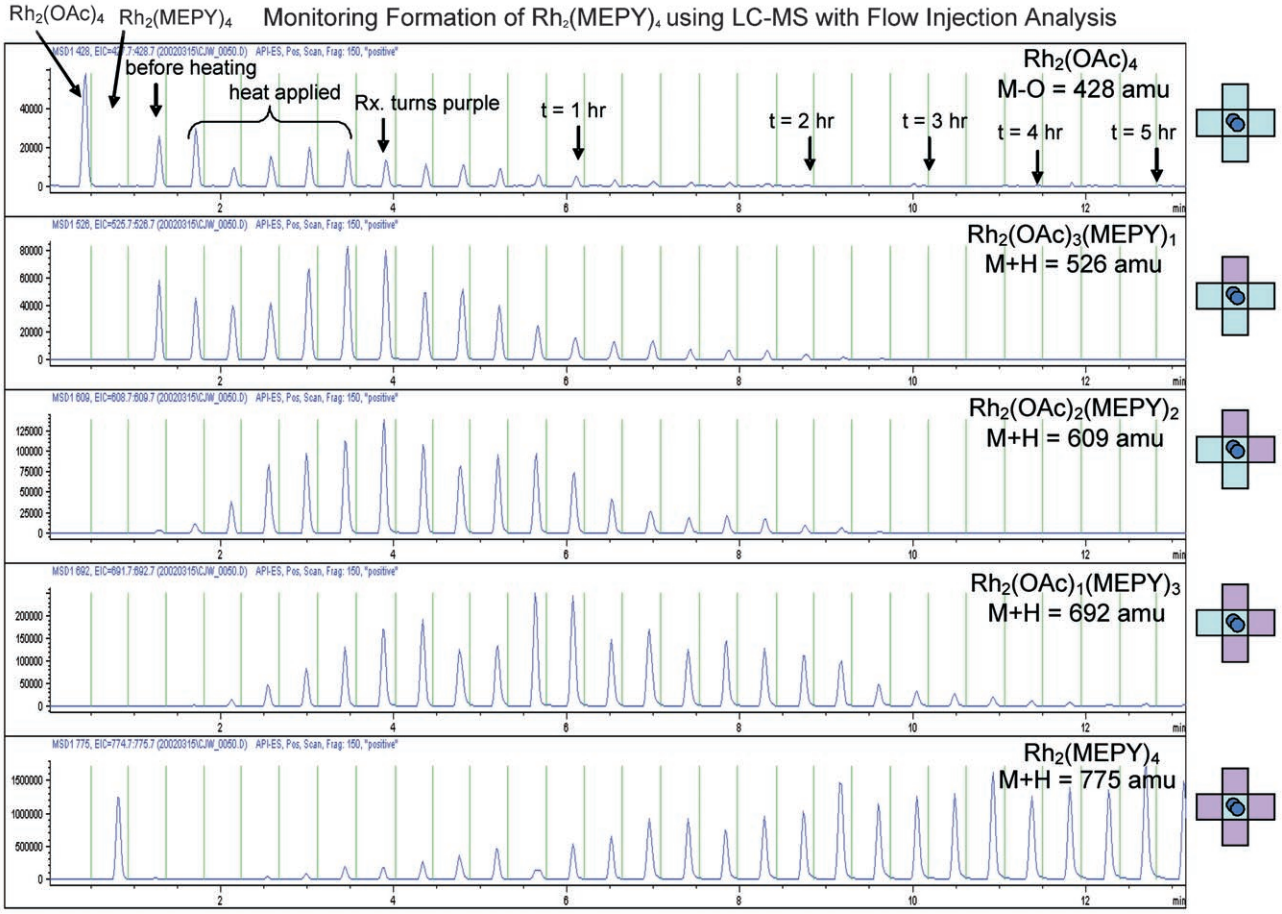


Figure 1. Monitoring the formation of $Rh_2(MEPY)_4$ using LC-MS with flow injection analysis. Results presented as extracted ion chromatograms at characteristic ions for each species. Analysis conditions: Agilent 1100 LC-ESI-MS FIA; Extend C18 (4.6 × 50 mm) column, flow rate 1.5 mL/min; 50% ACN/water with 2 mM ammonium formate, pH 3.5; V_{frag} 150.

would be slower than the first two. We were therefore surprised to see the relatively rapid appearance of product and virtually no significant rate difference in proceeding from the first through the fourth ligand substitutions.

There are, however, a number of disadvantages to the rapid FIA HPLC-MS approach for detailed reaction monitoring, for example, the inability to visualize the individual isomers for each species. Nevertheless, we have found this approach to be a generally useful tool for quick analysis, and in this instance it enabled us to confirm that reaction mixture aliquots containing the various intermediates on the pathway to $Rh_2(MEPY)_4$ were sufficiently stable to allow investigation over reasonable experimental timescales.

A more thorough reversed-phase HPLC analysis of $Rh_2(MEPY)_4$ reaction aliquots using a gradient elution revealed a number of peaks, with ICP-MS detection showing the presence of a number of different ^{103}Rh-containing species. Thus, the chromatogram of a reaction aliquot at t = 80 min reveals the presence of 12 different rhodium containing species (Figure 2). Subsequent analysis using ESI-MS detection allowed the composition of all peaks to be assigned (M + H = 526, 609, 692 and 775 amu for mono-, di-, tri- and tetra-MEPY species, respectively). A number of interesting observations can be drawn from the HPLC-ICP-MS chromatogram. First, twelve of the theoretically possible 15 components are visualized, with only two $Rh_2(OAc)_2(MEPY)_2$ isomers and one $Rh_2(OAc)_1(MEPY)_3$ isomer being either absent or unresolved. Surprisingly, all four possible isomers of $Rh_2(MEPY)_4$ are observed, despite the fact that all but the (*cis*-2,2) isomer had been dismissed as not formed or not isolated.[11]

There are several implications from these findings that have repercussions on our understanding of this catalyst system and shed new light on what have appeared to be discrepancies in the reporting of enantiomeric excesses from reactions that use different preparations of these chiral catalysts. The existence of other $Rh_2(MEPY)_4$ isomers helps to rationalize the observation that purification of the catalyst by crystallization is crucial to obtaining a single isomeric product with the desired enantioselectivity properties, and why variations in catalyst preparation or storage can lead to changes in enantioselectivity when the catalyst is used in a reaction.[16] Interestingly, we have argued previously that the (*trans*-2,2) isomer was not formed,[11,12] but the data clearly show that this isomer is a minor component of the tetrasubstituted $Rh_2(MEPY)_4$ composite.

Having an analytical method that distinguishes the component species in the formation of the $Rh_2(MEPY)_4$ catalyst affords us an excellent tool for monitoring the progress of the reaction over time. In addition, this analytical capability may prove helpful for understanding enantiocontrol in the use of these catalysts, since each isomer has its own reactivity and selectivity profile. As has been recently emphasized by Blackmond[15] careful reaction monitoring can be useful for deconvoluting the reaction system or for more exacting evaluation of reaction mechanisms. The ability to perform these studies using ICP-MS detection offers a distinct advantage over more conventional ESI-MS detection, in that ICP-MS detection is not subject to the matrix-related ion suppression/ion enhancement prob-

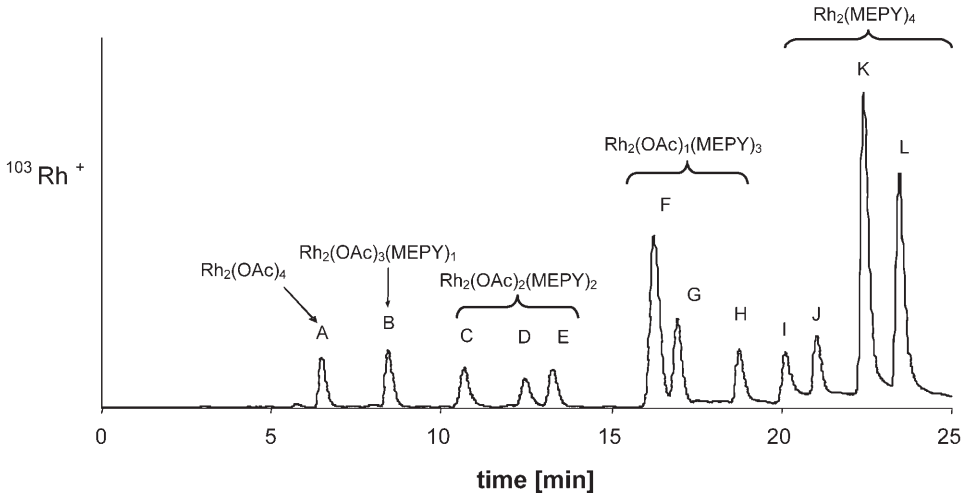


Figure 2. HPLC-ICP-MS chromatogram showing the presence of 12 different Rh-containing species in the formation of $Rh_2(MEPY)_4$: Conditions: Cadenza CD-C_{18} (4.6 × 250 mm) column; A: H_2O + 0.1% trifluoroacetic acid (TFA); B: 1:1 methanol/acetonitrile + 0.1% TFA. gradient: 20% B 0–12 min, 25% B 13–16 min, 30% B 17–25 min, 1 mL/min; Agilent 1100 HPLC system coupled with Agilent 7500cs ICP-MS through a custom-built interface consisting of eluent splitter, desolvating unit, and ICP-MS built-in peristaltic pump. ICP-MS conditions: power 1100 W; plasma gas flow 14.9 L/min; auxiliary gas flow 0.90 L/min.

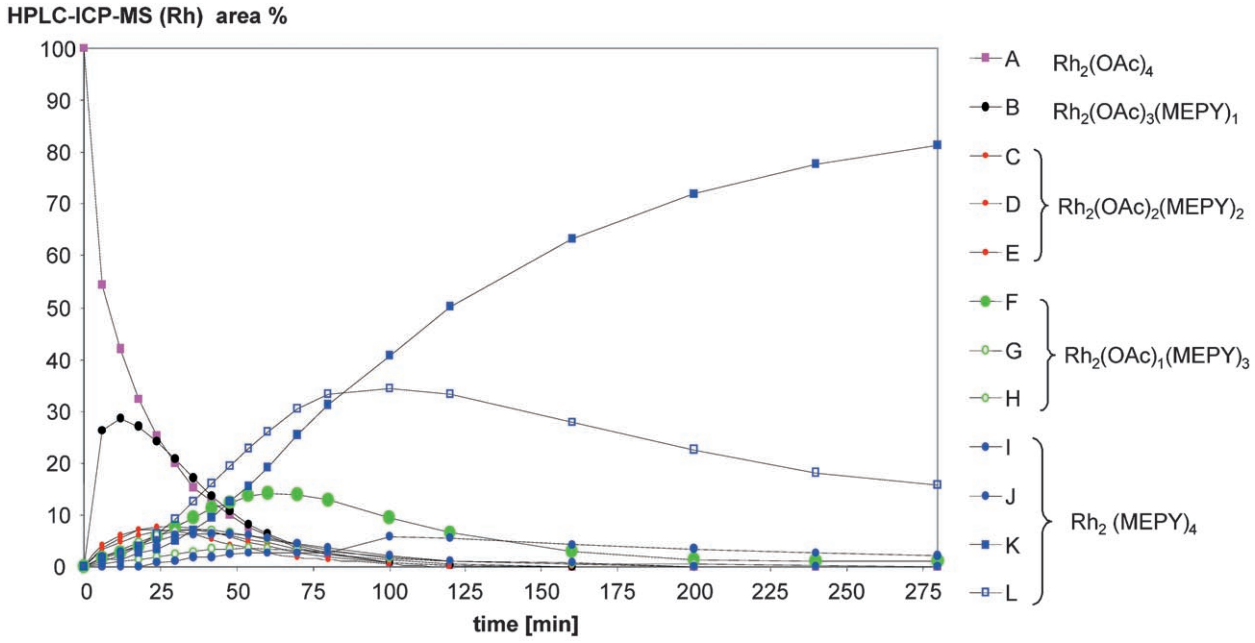


Figure 3. HPLC-ICP-MS evaluation of reaction time-course aliquots from the formation of $Rh_2(OAc)_4$ from rhodium acetate. HPLC-ICP-MS conditions as reported in Figure 2.

lems that make quantitation by ESI-MS notoriously difficult.

The results of the time course study, expressed graphically in Figure 3, reveal several interesting features. The decay of rhodium acetate (peak A) is seen to be quite rapid and, as in the previous study, we observe the mono-MEPY species (peak B) to rapidly grow in, then begin to decline at about 20 min. The various di-MEPY (peaks C, D and E) never substantially accumulate in the reaction mixture, whereas one of the tri-MEPY species, peak F, grows to a maximum of about 14% area at t = 1 h, before beginning to decline. Of greatest interest to us was the change of the various tetra-MEPY species over time. Two of the species (peaks I and J) are present at only low levels, whereas peak L is formed in substantial quantities, but appears to be gradually isomerized under the conditions of the reaction to the desired isomer (peak K). This observation is helpful in reconciling empirical observations concerning the relationship between reaction time and yield of crystallized (*cis*-2,2)-Rh2(MEPY)$_4$[14] and suggests an avenue for reaction monitoring so as to optimize yield.

The undesired isomer corresponding to peak L, which is presumably the (3,1)-Rh_2(5*S*-MEPY)$_4$ isomer in which the N and O positions of a single MEPY ligand have been flipped, can be seen to be critically important to a proper understanding of the catalyst formation reaction. The fact that the role of this isomer went undetected by conventional analytical techniques such as NMR, etc., while being easily distinguished using HPLC with ICP-MS and ESI-MS detection argues strongly for the important role that these analytical techniques may play in more completely investigating other metal-containing reaction systems. The study of other systems may be complicated by reactivity with solvent or oxygen, or by fast ligand exchange on the HPLC timescale. Nevertheless, simple modifications such as oxygen exclusion, the use of non-reactive eluents, or the use of fast chromatography or low temperature chromatography may be helpful in expanding the range of the approach.

Experimental Section

HPLC-ESI-MS was carried out using an Agilent 1100 MSD instrument (Agilent technologies, Wilmington, DE, USA). HPLC-ICP-MS was carried out using an Agilent 1100 HPLC instrument coupled to an Agilent 7500cs ICP-MS through a custom-built interface consisting of a PEEK Tee (0.50 mm, Upchurch Scientific, Oak Harbor, WA, USA) eluent splitter, an Aridus Desolvating Sample Introduction system (CETAC technologies, Omaha, NE, USA) and the ICP-MS built-in peristaltic pump. A portion of the eluent from the LC outlet was nebulized into a heated PFA spray chamber using a PFA microconcentric nebulizer, and transported to a heated microporous PTFE tubular membrane. Solvent vapor passed through the membrane and was removed by a stream of Ar gas, while the analyte continued through the membrane tube to the ICP-MS. Chromatographic data analysis was performed using the Agilent Plasma Chromatographic software.

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