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To be cited as: *ChemCatChem* 10.1002/cctc.201801637

Link to VoR: http://dx.doi.org/10.1002/cctc.201801637

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10.1002/cctc.201801637

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An integrated lab-on-a-chip approach to study heterogeneous enantioselective catalysts at the microscale

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Abstract: An integrated lab-on-a-chip enables the rapid analysis of heterogenized enantioselective organocatalysis at the microscale. A packed-bed microreactor was seamlessly integrated with a downstream chiral high pressure liquid chromatography (HPLC) functionality to study enantioselective transformations on a single microfluidic glass chip. Hyphenation to mass spectrometry allows for the rapid investigation of the selectivity and the substrate scope of microgram amounts of catalyst beads. Optimization of reaction conditions is possible with minimal reagent consumption and instant analytical feedback.

Heterogeneous catalysis is of central importance throughout the field of chemistry. The immobilization of catalysts to solid supports is particularly important with respect to enantioselective catalysis.[1–6] Different immobilization strategies involve a wide variety of molecular species and support materials.[7–10] The catalytic performance of an active material is strongly dependent on the nature of the catalyst, the solid support, the immobilization strategy as well as the use of various spacers to maintain the catalytic activity of the bound molecules.[11–13] While these supported catalysts are well suited for the intensified conditions accessible within continuous flow reactors,[14–22] finding the most suitable heterogenized catalyst materials for an intended application is a time and resource intensive process. Not only can the development of the respective materials be regarded as an art of its own, but the testing of the suitability and performance of the precious materials is often just as, or even more, time intensive. In flow chemistry, this elaborate process includes testing of various substrates, solvents, and additives with subsequent analysis of the reaction mixtures via complex analytical techniques.[23–25]

For enantioselective conversions, the determination of the enantiomeric excess is of central importance. Commonly this can be achieved by chiral HPLC, ideally in combination with mass spectrometry for accurate compound identification. The timescales for success, as well as the substantial consumption of resources such as chemicals and solvents, are quite dissatisfactory. Novel enabling technologies could improve the situation.[26]

Heterogeneous catalysis can be performed efficiently in miniaturized microfluidic devices, to significantly reduce the amount of catalyst required. Furthermore, the lower consumption of chemicals and solvents in such systems results in both economic and ecological benefits.[27–31] Typically, the analytical characterization is performed offline with traditional analytical equipment. As analysis then becomes the bottleneck in terms of process time[32,33], such an approach only partially addresses the challenges discussed above.

We developed various lab-on-a-chip approaches to study homogeneous catalysis in solution by integrating enantioselective catalysis and analysis on a single chip.[34–36] Such an on-chip integration of chemical transformation and analysis by seamlessly interconnected microfluidic channels minimizes reagent consumption, sample transfer times, and void volumes. This enabling technology offers new routes to study catalytic events at time and length scales hardly reachable with conventional technology. However, aside from interesting concepts combining catalysis and separation using on-column reaction gas chromatography as well as electrokinetic chromatography,[37,38] integrated lab-on-a-chip devices to study heterogeneous catalysts at the microscale are still missing.

Herein, we present an approach to study the catalytic performance of microgram amounts of silica-supported organocatalysts in a multifunctional microfluidic chip. We developed a lab-on-a-chip platform integrating a nanoliter-sized packed bed reactor which is seamlessly interfaced with a HPLC functionality using a chiral stationary phase for in-situ separation of enantiomeric products in the reactor effluent. By hyphenating the device to a mass spectrometer via electrospray ionization, separated compounds can be analyzed directly. The chip device includes two packed microcolumns, one for catalysis in flow and

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10.1002/cctc.201801637

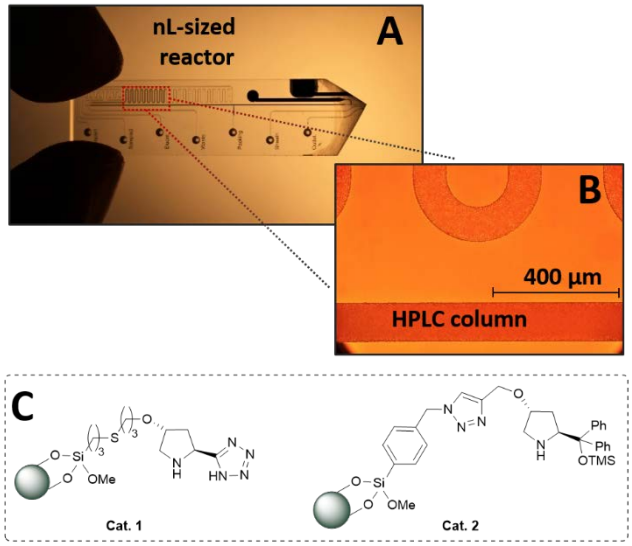

Figure 1. A Microfluidic chip with integrated packed-bed reactor and HPLC-column. **B** Magnified view of the two packings (catalyst beads on top and chiral stationary phase in the bottom). **C** On silica particles (Kromasil, 60 Å, 5 µm) immobilized Ley–Arvidsson–Yamamoto catalyst (**Cat. 1**, left) and the immobilized Hayashi-Jörgensen catalyst (**Cat. 2**, right), packed for the experiments inside the meandering structure of the chip.

the other for direct downstream HPLC enantioseparation (Figure 1).

The device was engineered based on our experience in designing glass chips incorporating HPLC columns.[39–42] A more complex device was realized by interconnecting two different packed-bed columns on a single microchip (see Supporting Information (SI) for details). The integrated chip device, requires significantly reduced amounts of catalyst when compared to traditional tube or capillary-based packed bed reactors. By avoiding interconnecting tubes and transfer lines void or swept volumes are eliminated for an unsurpassed efficient transfer of the reactor effluent to the HPLC functionality. The amount of catalyst is reduced by roughly a factor of a thousand (Figure 2).

Our column manufacturing process is compatible with the entire variety of commercially available stationary phases and many catalyst beads. Slurry packing of the particulate material, which is held in place by photopolymerized frits, is followed by sealing the packing channel via photopolymerization (for details see SI). By using the above mentioned slurry packing method, the organocatalyst-functionalized silica particles were trapped inside the meandering structure. The flexible column fabrication process allows for the integration of different sized packed bed reactor sizes of up to about 500 nL (maximal reactor volume). For our experiments, about half of this volume was filled with catalytically active particles. With a column exclusion factor of about 0.5, this leads to a packed bed void volume of about 100 to 150 nL. The reaction mixture is then immediately probed by the analytical unit of the chip to gain information about the reaction progress by quasi-real time online analysis (schematic explanation in the SI).

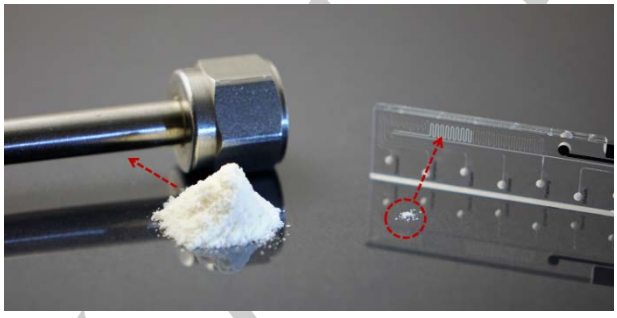

Figure 2. Comparison of the amount of catalyst approximately inside a conventional steel column flow reactor (left, 50 mm x 2.1 mm inner diameter) and the quantity inside the micro-flow reactor on chip (right, ca. 250 nL).

To evaluate this concept, the setup was used to study a model asymmetric Mannich-reaction[43,44] promoted by the silica supported Ley-Arvidsson-Yamamoto catalyst[45–47] (**Cat. 1**, 0.76 mmol·g^{-1} on porous silica with an outer diameter of 5 µm and a pore size of 60 Å). The heterogeneous pyrrolidinyl tetrazole organocatalyst **Cat. 1** was synthesized by thermal/photoinduced thiol–ene coupling starting from mercaptopropyl silica gel.[48] Afterwards, a solution of heptanal **1** (9.6 mmol·L^{-1}) and N-PMP-protected ethyl iminoglyoxylate **2** (1 mmol·L^{-1}) in acetonitrile (ACN) was introduced in the reactor unit via the sample inlets (Fig. 3 A). After passing the reaction mixture through the catalytic bed, the reaction outcome was probed using the integrated HPLC-column with MS detection. It was possible to investigate the effluent after a reactor residence time (RT) of less than 1 s (taking into account the void volume and a flowrate through the packed bed of 10 µL·min^{-1}) and clearly detect the formation of the syn-Mannich adduct **3** (m/z 322) in the chromatogram (Fig. 3 B, green line) with high diastereoselectivity (d.r. > 10:1) and excellent ee (>95%). These results were in good agreement with respective batch experiments on larger scale (see SI). In the chromatogram of the flow experiment, the unreacted imine **2** in the form of the corresponding aniline (m/z 124; blue line) was also

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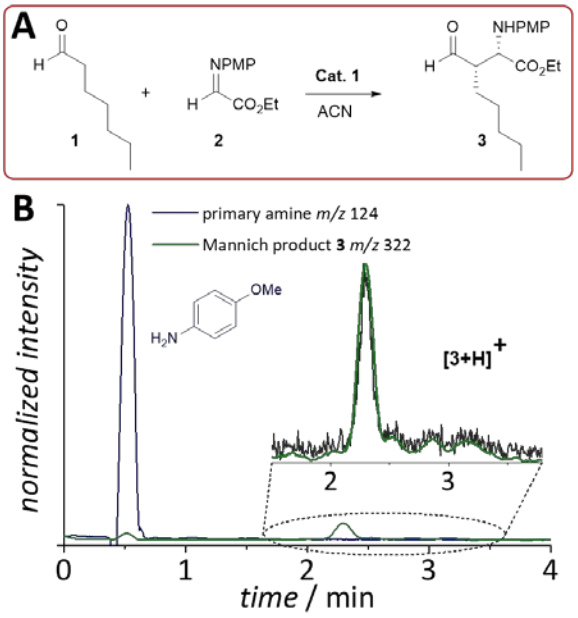

Figure 3. A Mannich-reaction catalyzed by the silica supported Ley-Arvidsson-Yamamoto catalyst. **B** Extracted ion chromatograms (EICs) for the separation after on-chip continuous flow synthesis. Product signal after a residence time of approximately 1 s (green line). The smoothed chromatogram (52 point Savitzky-Golay filter) is shown in the magnification of the product EIC. Reaction mixture: **1** (9.6 mmol·L^{-1}), **2** (1 mmol·L^{-1}), in pure ACN. Column: Chiralpak IG-3, particle diameter 3 µm, length 35 mm, mobile phase: 160 µL·min^{-1}, ACN/H2O (60/40 vol% with 0.1 vol% formic acid), sample flow: 10 µL·min^{-1}, ACN, pinch: 2 µL·min^{-1}, ACN/H_2O (55/45 vol% with 0.1 vol% formic acid). 10 bar elution pressure at the injection cross at a linear flow rate of 1.3 mm·s^{-1} over the column.

observed, which was formed by hydrolysis of **2** when in contact with the aqueous eluent solution in the HPLC functionality. Although the conversion in these micro-flow experiments were relatively low, it allowed a rapid assessment of the stereoselectivity of tiny amounts of the catalyst material.

After the successful proof-of-concept using a model catalyst system, the broader applicability of the approach was investigated. For this purpose, an analogous device was fabricated using other catalysts. Silica-immobilized Hayashi-Jörgensen catalyst[49,50] **Cat. 2** (0.18 mmol·g^{-1} on Kromasil 60-50-SIL) was incorporated inside the chip-reactor. This novel catalyst was synthesized from an azide-functionalized silica gel by adapting a known procedure[51] (see the SI). **Cat. 2** was utilized for studying the asymmetric α-amination reaction of aliphatic aldehydes **4-7** with dibenzyl azodicarboxylate (DBAD) **8** in a flow regime[52] (Fig. 4 A). In this set of experiments, very low conversions were observed under different flow conditions. In order to extend the residence time, the experiments were run in stop-flow mode by infusing and hydraulically trapping the reaction mixture in the packed bed reactor for a given time. The reaction mixture consisted of the aldehyde **4-7** (6.25 mmol·L^{-1}), DBAD **8** (1 mmol·L^{-1}) and acetic acid (0.62 mmol·L^{-1}) in pure ACN. Chromatographic analysis of the coupling of hydrocinnamaldehyde **7** with **8** lead to the α-amination product **12** after an overall reaction time of 20 min (Figure 4 B). This device also was equipped with a novel chiral HPLC-selector phase (Chiralpak IG-3), to separate the enantiomers of the product **12** (6%) present in the reactor effluent. The instant analytical feedback documented the poor stereocontrol by the chiral **Cat. 2** in the investigated process

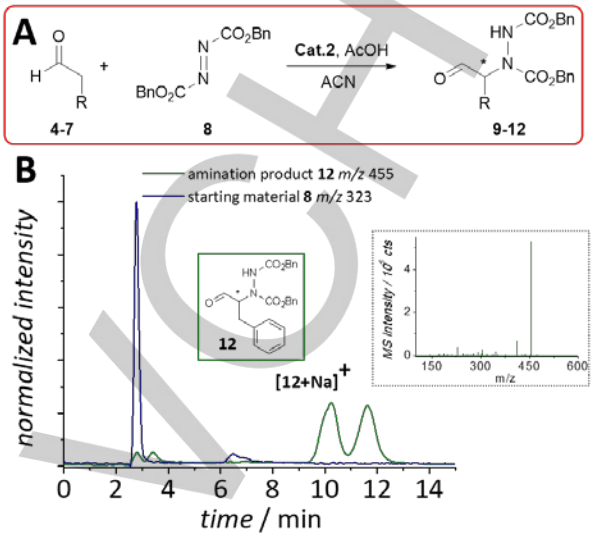

Figure 4. A Asymmetric α-amination reaction of aldehydes catalyzed by the silica immobilized Hayashi-Jörgensen catalyst. **B** EIC for the separation after on-chip synthesis using the stop-flow mode (extended residence time to 20 min). MS/MS-spectrum of the product peaks in the box. Reaction mixture: hydrocinnamaldehyde **7** (6.25 mmol·L^{-1}), **8** (1.25 mmol·L^{-1}), acetic acid (0.62 mmol·L^{-1}) in pure ACN. Column: Chiralpak IG-3, particle diameter 3 µm, length 35 mm, mobile phase: 160 µL·min^{-1}, ACN/H_2O (45/55 vol% with 0.1 vol% formic acid), sample flow: 10 µL·min^{-1}, ACN, pinch: 5 µL·min^{-1}, ACN/H_2O (60/40 vol% with 0.1 vol% formic acid). 12 bar elution pressure at the injection cross at a linear flow rate of 0.8 mm·s^{-1} over the column.

(Figure 4). The on-the-fly MS analysis allowed for the unambiguous identification of the product **12** at m/z 455 (insert of Fig. 4 B).

This stop-flow (or micro-batch) approach also allows for the impact of the residence time on product formation to be studied at the microscale, providing insights on the saturation capacity of the catalytic bed and consequently, the process productivity (Fig. 5, green curve). For this purpose, an external calibration was carried out using an authentic sample of **12** in the presence of a corresponding amount of **8** and acetic acid. Formation of the α-amination product **12** reached a plateau at about 50% yield. The same chip was operated to test the efficiency of the silica-supported Hayashi-Jörgensen catalyst **Cat. 2** in the formation of adducts **9-11**. From the analysis of the curves in Figure 5, the highest productivity resulted for the compound **11** obtained in 65% yield from the medium-chained aliphatic aldehyde **6**.

In summary, a novel approach integrating immobilized heterogeneous organocatalysis with HPLC-MS on a single microfluidic device, enables the rapid analysis of minute amounts of precious catalysts. After a first investigation of a highly stereoselective Mannich-reaction promoted by the silica supported Ley-Arvidsson-Yamamoto catalyst, the experimental setup was used to compare the efficiency of the silica immobilized version of the Hayashi-Jörgensen catalyst in a series of asymmetric α-amination reactions using a stop-flow reaction technique. It was possible to reuse a single chip over a time period of a couple months and the determined diastereo- and enantio-

selectivities were in good agreement with batch or up-scaled flow experiments, as documented in the supporting information. Given the possibility to easily change both stationary phases, namely the chiral catalyst and selector, we believe that our devices may serve not only as powerful screening tools by offering new insights on supported heterogeneous catalysis, but also for mechanistic elucidations, with key benefits like minimum consumption of resources and ease of automation.

Acknowledgements

This work was supported by the ESF (European Social Fund), Studienstiftung des dt. Volkes (PhD scholarship J. J. Heiland), Chiral Technologies Europe (esp. Dr. Pilar Franco, providing support and chiral stationary phase material), the Max-Planck-Institute, and the Deutsche Forschungsgemeinschaft (DFG) for funding (FOR 2177).

Keywords: Supported catalysis • Asymmetric catalysis • Microreactors • Liquid chromatography • Lab-on-a-chip

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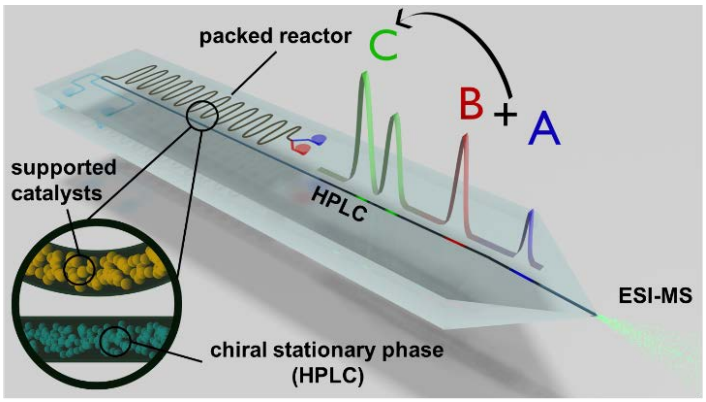

A rock-solid approach combines supported catalysis and chip-based HPLC.

Combining a packed bed microreactor and miniaturized HPLC on a single chip-based device enables rapid insights into asymmetric heterogeneous catalysis while producing minimal waste and requiring only minute amounts of immobilized organocalysts.

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