Lab on a Chip

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Asymmetric Organocatalysis and Analysis on a Single Microfluidic Nanospray Chip**

*Stefanie Fritzsche, Stefan Ohla, Petra Glaser, David S. Giera, Marcel Sickert, Christoph Schneider, and Detlev Belder**

The miniaturization of chemical processes onto so-called lab-on-a-chip devices has gained significant importance in different fields of chemistry. Besides the advantages of enhanced portability, reduced reagent consumption, and improved safety, a characteristic feature of miniaturized platforms is the possibility to achieve higher reaction and analysis rates.[1] From their roots in analytical sciences, microfluidic systems have received much attention over the past decade. At present, microfluidics is becoming increasingly popular in inorganic and organic chemistry, as syntheses are performed on chip-based microreactors[2] or capillary-based microflow reactors.[3] While diverse reactions have been performed in microfluidic chip devices with impressive results,[4] the analytical characterization is, however, usually carried out off-chip by conventional macroscopic instruments. However, this approach does not exploit the promise and the full potential of chip technology, namely, the integration of different functionalities such as chemical synthesis and analysis on one single device. Thus, it is desirable to develop, in analogy to microelectronics, integrated chemical circuits[5] as new chemical tools, for example, for catalyst screening[6] or for online monitoring of biological processes.[7]

In previous work we demonstrated a first approach for integrating chemical reactions and analysis on a single microchip for the screening of enantioselective biocatalysts.[8] Nevertheless, this system was limited to aqueous media and native fluorescent molecules.[9]

Therefore we intended to develop an advanced chip system with a wider applicability in synthetic chemistry, including the utilization of non-aqueous reaction media and a more general detection system. In this context, the coupling to mass spectrometry appears to be very attractive,[10] as it provides additional structural information for substance identification.

To demonstrate our concept, we focused on organocatalysis, which has been one of the most innovative research fields in synthetic chemistry over the past years.[11] Recently, Odedra and Seeberger[12] described a first miniaturized approach, performing organocatalysis in a microfluidic flow reactor chip. The reaction products were, however, analyzed offline by traditional HPLC.

Herein we present the, to our knowledge, first asymmetric organocatalytic reaction on a single chip with integrated analysis. As a model system we chose the enantioselective vinylogous Mannich reaction published in 2008, which is catalyzed by chiral phosphoric acid.[13] The alcoholic solvent mixture and the nonfluorescent reaction products in this synthesis are quite challenging for chip integration. Thus, it was necessary to adapt the reaction media to the aqueous separation electrolyte on-chip to enable an undistorted electrophoretic analysis of the reaction products.

Therefore, we developed a new microfluidic chip design containing different functionalities, including a reaction structure for the organic synthesis, a structure for aqueous dilution of the reaction mixture, a cross section for injection, and a separation channel for chip electrophoresis. Furthermore, the chip layout includes an integrated nanoelectrospray emitter with makeup-flow channels for dead-volume free coupling to mass spectrometry at the end of the separation channel.[14] A schematic drawing of the chip with 50 μm wide channels and a photo is shown in Figure 1.

Reaction and analysis processes on the single chip were carried out as follows: initially, the reaction structure was filled with the alcoholic solvent mixture for synthesis; then the remaining structure was replenished with the aqueous separation electrolyte. Afterwards, each reactant solution

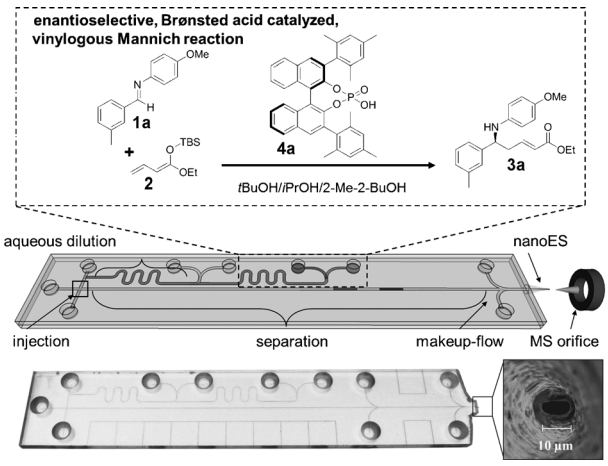

Figure 1. Schematic drawing (top) and photograph (bottom) of the chip layout with reaction, separation, and mass spectrometric detection by nanoelectrospray (nanoES) ionization.

[*] S. Fritzsche, S. Ohla, P. Glaser, Prof. Dr. D. Belder
Institute of Analytical Chemistry, University of Leipzig
Linnéstrasse 3, 04103 Leipzig (Germany)
E-mail: belder@uni-leipzig.de
D. S. Giera, Dr. M. Sickert, Prof. Dr. C. Schneider
Institute of Organic Chemistry, University of Leipzig
Johannisallee 29, 04103 Leipzig (Germany)

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(8 μL) was placed in the respective microvial of the chip, and the reaction in the mixing structure was initialized by applying pressure and voltages. The organic reaction phase was subsequently diluted with the separation electrolyte consisting of 0.1 vol% acetic acid and 25% methanol (v/v) in the second serpentine structure. Thereafter, the aqueous alcoholic reaction mixture was guided to the separation channel by applying a voltage-controlled pinched injection.[15] The separation was carried out by electrophoresis, and at the end of the separation channel, the analytes were transferred into the mass spectrometer via the integrated nanospray emitter. Further information and details of the working principle are described in the Supporting Information.

The MS electropherogram in Figure 2 exemplarily shows the result of the integrated process with on-chip catalysis and analysis. Since the reaction was carried out in 3 min and the

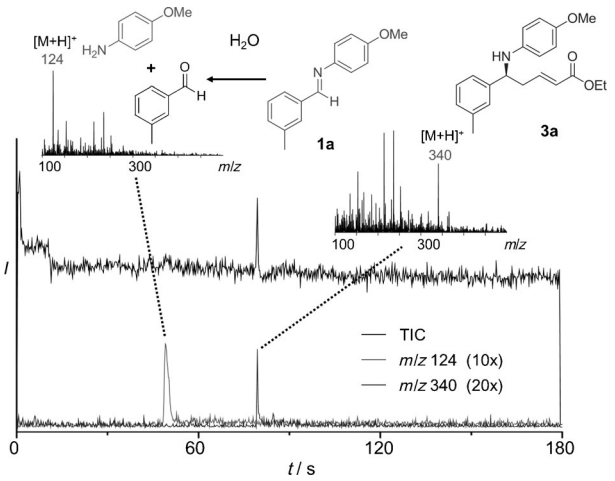


Figure 2. Total ion electropherogram and extracted mass traces with respective mass spectra of the on-chip organocatalysis. TIC = total ion current.

separation with MS-detection was performed in 1.5 min, the total time for synthesis and analysis took less than 5 min. With the help of the mass spectra, the reaction product **3a** as well as the starting material **1a** could be clearly identified, whereby the imine immediately hydrolyzes in aqueous media and was detected as the corresponding primary amine.

After successful integration of on-chip catalysis and analysis, the next challenge was the enantiomeric separation of the reaction product in order to determine the *ee*-value of the synthesis. This can be achieved by chiral modified chip electrophoresis.[16] In automated capillary electrophoresis experiments, the chiral selector hydroxylproyl-γ-cyclodextrin was found to be a suited additive for the separation electrolyte. However, the transfer to the chip scale was difficult because of the short separation length of 7.5 cm. Thus, we developed a second microchip with an elongated separation length of 23 cm to facilitate the separation of the enantiomers. To our knowledge, this enantiomer separation is the first to be performed on a microchip with mass spectrometric detection.

The organocatalytic synthesis was carried out by pipetting and mixing the reactant solutions directly in one microvial. The resulting reconstructed electropherogram of an asymmetric on-chip catalysis with enantiomer separation as well as a schematic drawing of the second microchip is depicted in Figure 3.

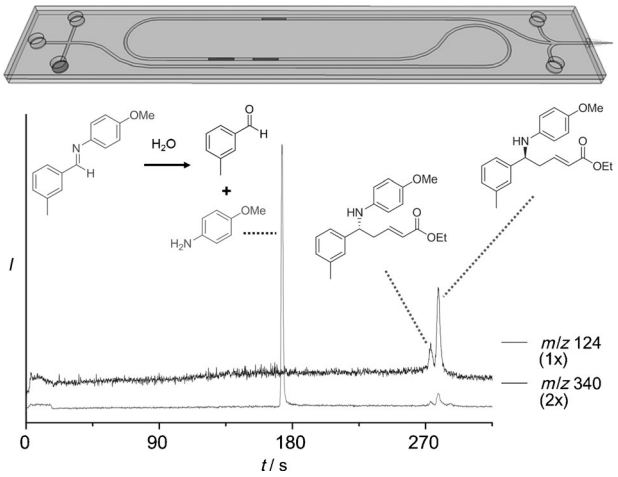


Figure 3. Electropherogram reconstructed from the extracted mass traces showing the chiral separation of on-chip reaction products.

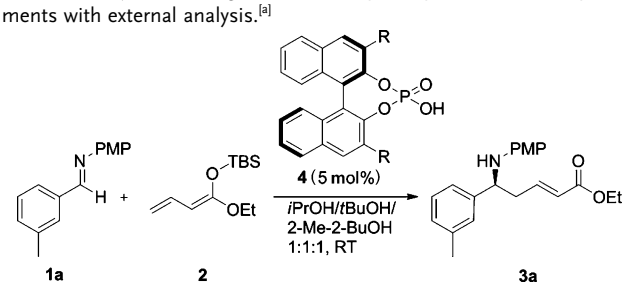
This setup enabled the rapid determination of the enantiomeric excess of an asymmetric reaction. For the example given in Figure 3, we obtained 55% *ee*. The reaction yields can be evaluated by adding an internal standard to ensure exact quantization. To test our system as a screening tool, we performed the reaction with different binol-based phosphoric acids and compared the results to traditional batch experiments with external HPLC analysis (Table 1).

The given *ee* values for chip experiments are mean values of triplicate determination with standard deviation of less than 5%. These data show that the enantioselectivity induced by the investigated catalysts can be reliably determined. In comparative batch experiments, it was found that the enantioselectivities can be considerably increased by reducing the temperature to −30°C (Table 1). Furthermore, *ee* values above 90% could be obtained in optimized solvent mixtures.[13e]

In comparison to macroscopic synthesis the microfluidic experiments were performed at adapted conditions to increase the robustness of the microfluidic processes. Nevertheless, the data show that rapid and unequivocal identification of the best hits is possible. Beside the variation of the catalyst, we also investigated different imines, which resulted in good agreement between traditional macroscopic and chip-based synthesis. As an example, the reaction of (*E*)-*N*-(4-ethylbenzylidene)-4-methoxyaniline under catalysis of 3,3′-dimesityl-substitued binol-based phosphoric acid is shown in Scheme 1.

The high process speed achievable with our microfluidic nanospray chips allows us to monitor reactions instantaneously by mass spectrometry. As model system we chose the organocatalytic transformation of **1a** from Figure 1. For that purpose we employed a microchip with several integrated makeup-flow channels allowing inflows at two positions (2.1

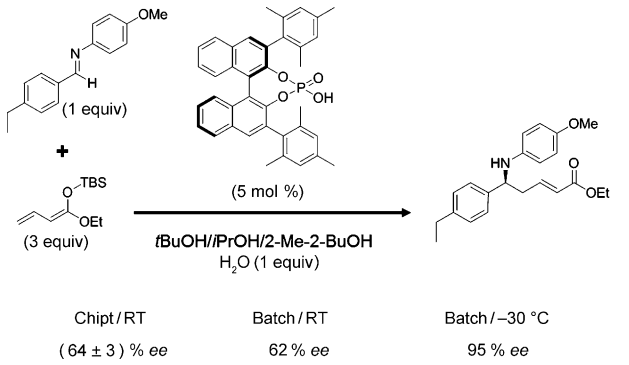
Table 1: Catalyst screening on a microchip compared to batch experiments with external analysis.[a]



1a 2 3a

No.	R	4	Method	*ee* [%][b]
1	2,4,6-$(CH_3)_3C_6H_2$	**4a**	chip/RT	55 ± 3
			batch/RT	62
			batch/−30 °C	73
2	2,4,6-$(iPr)_3C_6H_2$	**4b**	chip/RT	40 ± 4
			batch/RT	26
			batch/−30 °C	48
3	4-*t*Bu-2,6-$(CH_3)_3C_6H_2$	**4c**	chip/RT	66 ± 4
			batch/RT	76
			batch/−30 °C	85

[a] Reaction conditions: **1a** (1 equiv), **2** (3 equiv), **4** (5 mol%), room temperature, H_2O (1 equiv), chip: 22 mM in *i*PrOH/*t*BuOH/2-Me-2-BuOH (1:1:1), batch: 0.1 M in *i*PrOH/*t*BuOH/2-Me-2-BuOH (1:1:1). [b] On-chip with chiral selector, batch results by HPLC on chiral stationary phases.



Scheme 1. Asymmetric organocatalytic conversion of (*E*)-*N*-(4-ethylbenzylidene)-4-methoxyaniline with comparison of *ee* values from chip and batch experiments.

and 0.5 cm) ahead of the nanospray needle (Figure 4). As depicted, the reaction was initialized in the upper inlets and the reaction mixture was electrokinetically pumped toward the nanospray emitter, with subsequent dilution at the front channels to enhance the ionization. The analysis in the mass spectrometer was achieved within one minute after the reaction start. We clearly observed the contact ion pairs of **4a** with **1a** and **3a** as well as the protected catalysts TBS-**4a** (TBS = *tert*-butyldimethylsilyl). Although ion suppression is reduced in comparison to traditional electrospray,[14b] an exact quantification is, of course, difficult. However, the detected species were in good agreement with the postulated reaction mechanism.[13g] Beside the detection of **3a** and **1a**, the hydrolysis of **1a** to **5** was also monitored. Further optimization of the chip layout by shortening channel lengths, pressure assistance, and the utilization of fast mass spectrometers[17]

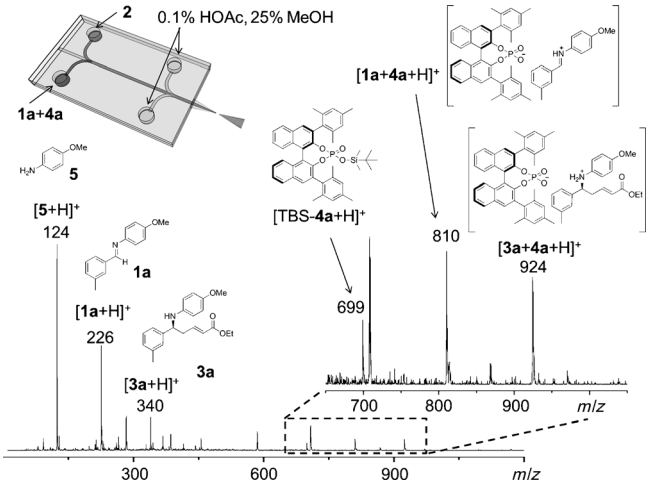


Figure 4. Investigation of reaction mechanism by microsynthesis-nano-electrospray mass spectrometry.

should reduce the residence time down to the subsecond scale, thus enabling the detection and identification of short-lived species.

We demonstrated the first combination of enantioselective organocatalysis, enantiomer separation, and mass spectrometric detection on a single microchip. This chip system was successfully applied to screen catalysts in asymmetric synthesis. The obtained results showed a very good correlation with comparative batch experiments. Additionally, the first enantiomer separation on a microchip with mass spectrometric detection could be realized. Furthermore, the utilized microchip design offered the possibility to study reactions by mass spectrometry directly after initialization. This feature enables the detection and identification of reaction intermediates and thus exhibits great potential for the investigation of reaction mechanisms.

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