On-line monitoring of a microwave-assisted chemical reaction by nanolitre NMR-spectroscopy†

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We report the use of a nanolitre nuclear magnetic resonance (NMR) spectroscopy microfluidic chip hyphenated to a continuous-flow microlitre-microwave irradiation set-up, for on-line monitoring and rapid optimization of reaction conditions.

Microwave-assisted organic synthesis (MAOS) has gained wide attention as alternative heating mode in fields such as organic chemistry and bioanalytical applications, medicine and high-throughput chemistry.[1] Microwave-assisted continuous flow organic synthesis (MACOS) has been introduced to minimize elaborate sample-handling, and combines the unique heating mechanism of microwave irradiation with the safety, reproducibility, facile automation and process control conditions of continuous flow techniques.[2] Recently, the development of MACOS with microreactors has further boosted the performance of microwave-assisted capillary organic synthesis, with significant impact on reaction rates and scale up.[3] Raman spectroscopy has been used for "*in situ*" monitoring of microwave reactions.[4] UV/vis spectroscopy has been coupled with a microwave reactor for continuous-flow on-line detection, using a reactor coil of 159 μL for a linear alcohol derivatization.[5] However, a chemical selective method for on-line monitoring of MACOS has not been implemented yet and "*ex situ*" analysis is usually carried out after product collection, that slows the overall synthetic process.[2,3]

We here report the design and implementation of a microlitre-microwave reactor hyphenated with a custom-made nanolitre-NMR set-up, comprising a less than 2 μL reaction volume for the microwave flow cell and a 6 nL detection volume microfluidic NMR chip (Fig. 1). The detection volume of the NMR chip was chosen to be much smaller with respect to the microwave cell to have a dynamic range for the monitoring of different reaction times whilst keeping the flow rate and correlated reaction conditions constant. In this way the reaction volumes submitted to microwave heating at different irradiation times, *i.e.* at different starting positions in the capillary, can be analysed within one and the same on-flow experiment. To prove the microwave–NMR concept for optimization of reaction conditions, the well-known Diels–Alder cycloaddition of 2,5-dimethylfuran and dimethylacetylene dicarboxylate was chosen as model reaction. (see ESI† and Scheme S1 for details on the reaction).

The inherent low sensitivity of NMR spectroscopy has for long thought to be a limiting factor for volume and mass-limited sample analysis. However, as the signal-to-noise ratio (SNR) increases with decrease in coil diameter, the microcoil concept can overcome this limitation.[6] Although planar coils have limitations regarding filling factor when compared to other small-volume NMR-coil designs, *e.g.* solenoidal microcoils,[6] stripline,[7] or microslot,[8] the planar-coils have several advantages: they are manufactured by rather straightforward and standardized microfabrication techniques, they allow a well-controlled geometry of the coil, as well as a precise positioning of the RF-transceiver coil and sample, and allow the straightforward integration with microfluidic connections and devices. We have recently designed microfluidic chips for ^{1}H NMR[9] and ^{19}F NMR[10] spectroscopic applications, to study supramolecular assemblies and interactions at picomole concentrations. We here present the design of a 6 nL NMR-chip with integrated microfluidic connections for flow-through implementation with a microlitre-volume microwave-irradiation set-up (see Fig. 1).

For an effective integration of all components of the setup, the two major components were designed and optimized, separately. First, the microfluidic NMR-chip was fabricated and validated to work at 7.05 T (300 MHz ^{1}H Larmor frequency), to obtain the required high sensitivity and resolution for chemical identification of (sub)nanomol quantities

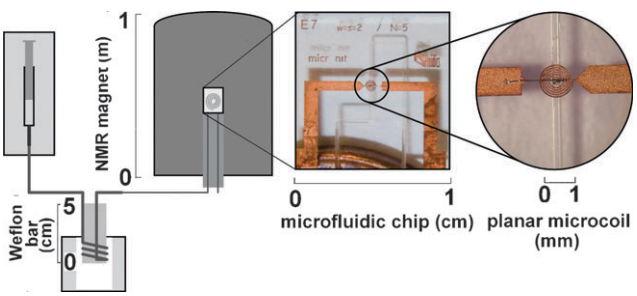

Fig. 1 Scheme of the hyphenated syringe–microwave–NMR set-up (left), with zoom on the microfluidic NMR chip (middle), and further zoom on the integrated planar radiofrequency transceiver microcoil on top of the 200 micron wide fluidic channel. The bars with different units indicate the different dimensions of the set-up.

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of material. For the microlitre microwave reactor, on the other hand, a commercial reactor (Resonance Instruments, Inc. Model 521) fabricated for batch synthesis purposes, was customized replacing the sample vial by a designed flow-cell, reactions were monitored in off-line mode in order to establish the performance of the modified reactor.

Thus, having optimized the performance of the two sub-systems, an effective integration of the microwave reactor with the NMR microfluidic chip was done. The used microwave reactor has a cylindrical cavity applicator separated from the microwave magnetron *via* an output coaxial cable, allowing the positioning of the reaction vessel in close proximity, *i.e.* within the strayfield, of the NMR magnet. A capillary (fused silica) wound in and out of the cavity and wrapped around a Weflon™ bar defined a reaction volume exposable to microwave irradiation of 1.6 μL. For the interfacing to the detection system, the capillary which forms the reaction cell (fused-silica capillary length 55 cm; OD 349 μm, ID 100 μm) is connected to the capillary attached to the NMR-chip (fused-silica capillary, length 62 cm, OD 109 μm, ID 40 μm). A high-force pump with SGE gas chromatography syringe controlled the flow in the system. The NMR-chip was placed on a custom-built probe-holder for optimized positioning and handling of the NMR-chip inside the bore of a (Varian) 7.05 T magnet and connected to the spectrometer allowing ^{1}H NMR spectroscopy at 300 MHz. The minimal thickness and long length of the capillary tubing between the microwave cavity and the NMR chip allow the cooling of the reaction sample to RT before it reaches the detector. This was proven by monitoring the negligible change in the temperature-sensitive NMR signals of a methanol sample under different heating powers of the microwave and under different flow conditions.

The performance of the MW–NMR system was first explored using stopped-flow measurements. Conversion values for the cycloaddition reaction (percentage of dienophile converted into cycloadduct), as a function of temperature, for a fixed reaction time (Table S1, ESI†), and as function of reaction time at constant temperature (Table S2, ESI†) were obtained, indicating the designed set-up to enable the readily monitoring of microwave-assisted chemical reactions by NMR spectroscopy.

Consecutively, on-flow measurements were carried out, detecting the reaction product formed as a function of the microwave heating. The microwave reactor was set up at three different temperatures (125, 135 and 150 °C) and reagent conversion was determined as a function of exposure time to microwave irradiation. As the microfluidic chip has a lower detection volume (6 nL) than the microwave reaction volume (1.6 μL), the latter can be divided in different zones for analysis. Considering the fact that the onset of the microwave, *i.e.* the time needed to attain the maximum heating power, is only a few seconds (2 s for 125 °C up to about 5 s for 150 °C), switching on the irradiation power when working under flow conditions, every small portion of the reaction volume is exposed to microwave irradiation for different times. Consequently, multiple data points can be sampled subdividing the microwave reaction volume in volumes between 6 nL and 1.6 μL. Depending on the reaction conditions chosen (*e.g.* concentration, flow rate, temperature) a number of data points can be obtained (conversion *vs.* reaction time) from one experiment (Fig. 2), varying the flow speed and acquisition parameters in the ^{1}H NMR experiment (*e.g.* 100 accumulated scans of 0.5 s at a flow rate of 0.21 μL min^{-1} results in a detection volume of 175 nL).

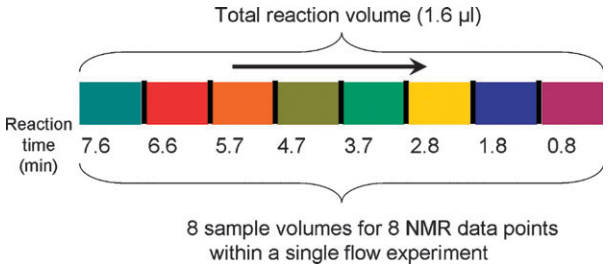

Fig. 2 Scheme representing the eight sample portions of the reaction volume exposed to the microwave irradiation for different times (0.8, 1.8, 2.8, 3.7, 4.7, 5.7, 6.6, 7.6 min), and analyzed successively (see Fig. 3) in a single <2 μL flow experiment. The arrow indicates the direction of the flow.

Fig. 3 shows the ^{1}H NMR spectra for different reaction times (Fig. 2) at a reaction temperature of 150 °C. The NMR peak for the reaction product (3.5 ppm) increases in intensity as the reaction time increases. The NMR peaks for the reagents (3.3 ppm for the dienophile and 5.8 ppm for the diene) decrease in intensity as the reaction time increases. Spectrum 1 and 8 correspond to 14 and 48 nmol of reaction product, respectively, in a total sampled volume of 175 nL (for a reagent concentration used of 0.75 M).

Fig. 4 shows the conversion on the cycloaddition product for different reaction times and at three different temperatures. The data for a certain temperature are obtained from a single continuous-flow experiment and data points correspond to different times of exposure to microwave irradiation (Fig. 2 and Table S3, ESI†). For this reaction, the set-up was optimized to work close to the limit of detection on the lowest conversion data point (Fig. 4, 125 °C, 1 min datapoint), which corresponds to 7% conversion, 9 nmol absolute amount of product, in a total sampling volume of 175 nL.

As a control experiment we have performed the Diels–Alder reaction using the MW–NMR set-up described above, but

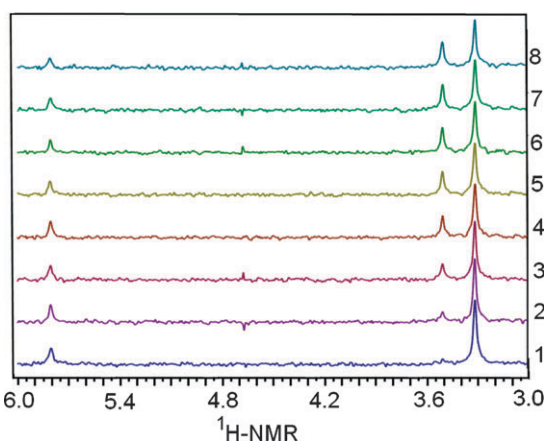

Fig. 3 Expansion of the ^{1}H NMR spectra obtained at 150 °C for different reaction times (from 0.8 to 7.6 min from bottom to top). Data are obtained on-flow (0.21 μL min^{-1}).

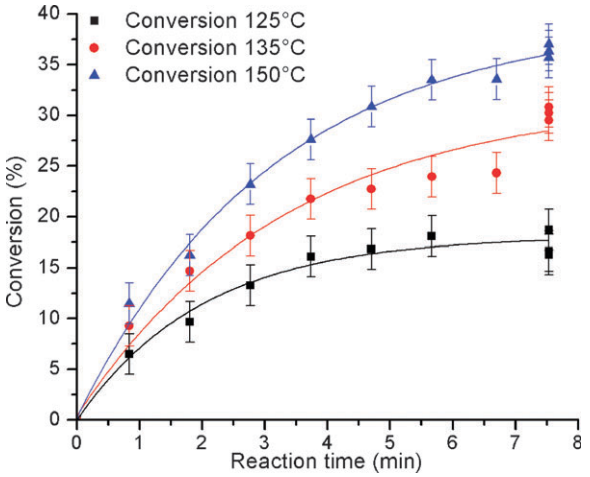

Fig. 4 Conversion (%) of the cycloaddition reaction product *vs.* reaction time for three different temperatures (125, 135 and 150 °C).

applying heating of the 1.6 μL reaction volume part of the capillary with a conventional heating plate set-up. For practical reasons the magnetic heating plate can not be put close to the NMR magnet, and fast switching between different temperatures with these small volumes could not be done either; therefore, several μL of reaction product were collected, diluted with toluene-d_8 and measured in a conventional NMR tube. At a maximum reaction time of 7.6 min the same conversions of the starting compounds were observed as obtained with the microwave heating, proving the MW–NMR set-up not to have any adverse effects on the outcome of the reaction. At the same time, performing the control experiment clearly illustrated the significant advantages of the MW-heating set-up, allowing the positioning close to the NMR magnet with concomitant short capillaries, and therefore reducing dead volumes and detection times, the on-line and on-flow detection capabilities, the facile integration with a small-volume NMR chip, the fractionated sampling of the reaction volume allowing multiple data point collection in one constant-flow experiment, and fast temperature switching.

In conclusion, we report for the first time the coupling of a microwave reactor to an NMR probe. We have proved the feasibility of on-line monitoring and optimization of a microwave activated reaction by small-volume NMR techniques. In accordance to the principles of Green Chemistry, the optimization of a microwave-assisted chemical reaction has been performed on a very small reaction volume, in short time, diminishing the quantity of waste produced (*e.g.* solvent), with an increased safety and consuming very low amounts of reagents, solvent and energy. Moreover, the current set-up works with standard, non-deuterated, solvents, which on the one hand further lowers the costs, and on the other hand, yields representative data for future scale up purposes, where for obvious reasons deuterated solvents are not used. Work is underway to broaden the scope of the set-up in the performance of high throughput chemistry with the development of a continuous-flow screening system. Also possible applications in the area of dipolar nuclear polarization sensitivity-enhanced NMR are being investigated.

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