J|A|C|S COMMUNICATIONS

Published on Web 03/25/2009

# A Microfluidic High-Resolution NMR Flow Probe

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NMR spectroscopy is an indispensable technique in chemistry, biology, and medicine. Despite its extreme site selectivity, which makes it by far the most information-rich analysis technique for molecular structure determination, NMR is seldom the method of choice for analysis of mass-limited samples because of the intrinsically low sensitivity. Since the absolute sensitivity of an NMR instrument for small sample volumes can be improved by decreasing the diameter of the detection coil, this miniaturization has been studied for more than a decade.[1−4] An improvement in terms of sensitivity and resolution using microcoil technology is very well possible, but the trade-off in terms of reduced resolution has impeded its fast incorporation into mainstream NMR analysis. Besides spectroscopic problems, micro- or nanoliter sample handling becomes difficult in a standard NMR setup. Small samples are ideally handled in microfluidic systems, which are available nowadays at a very high integration level. The implementation of microcoils in such a microfluidic system would offer the possibility of studying reaction kinetics.[5]

Currently, NMR on small liquid samples at room temperature is performed using small solenoids wrapped around a capillary[1] or planar coils on glass chips containing microfluidic channels.[2,6] Solenoid coils are limited in scalability, as they can hardly be produced with diameters less than 300 $\mu$m,[7] whereas planar coils are scalable but show lower signal sensitivity because of the inefficient contribution of the outermost windings to the radio frequency (rf) field.[8] More importantly, these approaches suffer from spectral resolution problems, mainly induced by the nearby windings of the microcoil that distort the static magnetic field.[6] To overcome these problems, we introduced a new NMR "coil", the stripline. This stripline design has an intrinsically higher sensitivity than solenoid and planar coils, as was demonstrated for both liquids and solid samples.[8a] A microfluidic chip based on this resonator showed superior spectral resolution (line width of <1 Hz) in pure ethanol without the use of additional susceptibility matching.[8b]

Here we describe a flow probe (Figure 1) based on this resonator that allows in situ monitoring of the kinetics of reactions performed in microreactors at full NMR resolution. The chip is positioned vertically in an aluminum tube and connected capacitively to the rf circuit. A fused silica capillary (length 200 mm, ID 100 $\mu$m) is used to fluidically connect the NMR chip to the microreactor. Glass microreactors (Micronit)[9] can be placed in a custom-built chip holder, which is mounted on top of the probe. Syringe pumps are connected to the reactor through FEP tubing to control the flow in the reactor. A detailed analysis of a $^1$H ethanol spectrum yielded a line width of 0.7 Hz. The line width at 0.55% is ~30 Hz, which is larger than that of commercial 5 mm probes but significantly smaller than those reported for other $\mu$NMR probe heads, including microslot-based probes.[3,10] The rf homogeneity is a further critical factor in pulse sequences that are more complex than a single pulse. In this design, the $A_{180°}/A_{90°}$ ratio amounts to 76%.

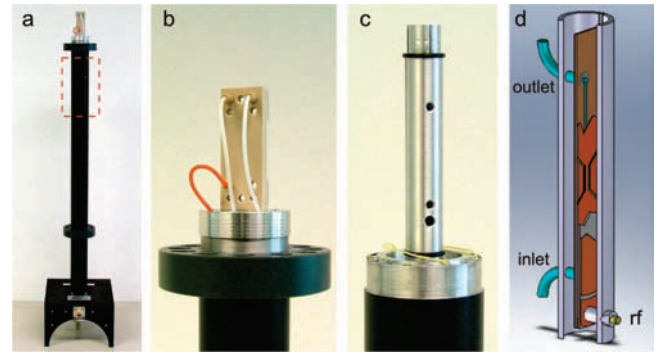


***Figure 1.*** (a) The custom-made microfluidic probe. The dashed line indicates the position of the NMR chip. (b) Close-up view of the microreactor holder mounted on top of the probe. (c) Close-up view of the stripline chip holder. (d) Schematic representation of the mechanical arrangement of the microfluidic chip in the holder.

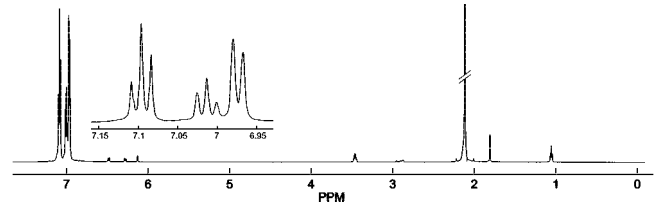


***Figure 2.*** Spectrum recorded during carbamate formation at flow rates of 24.46 and 0.54 $\mu$L/min for TDI and ethanol, respectively. Only resonances of solvents and reactants are visible because of the short reaction time (9 s) and the low TDI concentration. Inset: toluene aromatic peaks with all of the *J* couplings resolved, indicating the high-resolution performance.

To show the typical resolution, Figure 2 displays a spectrum recorded in flow (25 $\mu$L/min) during carbamate formation from toluene diisocyanate (TDI) (0.5 M in toluene) and pure ethanol. Since the high resolution is maintained, typical reactant or product multiplets can easily be identified. The probe allows the study of reactions in a time range from several seconds to 30 min, depending on the flow rate; the lower limit is determined by the volume of the tubing connecting the microreactor with the NMR detector chip, which is 4.5 $\mu$L in the current implementation.

Figure 3 shows two spectra obtained during real-time monitoring of the acetylation of benzyl alcohol with acetyl chloride in the presence of *N,N*-diisopropylethylamine (DIPEA) at different reaction times. These experiments allowed monitoring of the conversion over time, which was shown to accumulate to 70% in 3 min.

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10.1021/ja900389x CCC: $40.75 

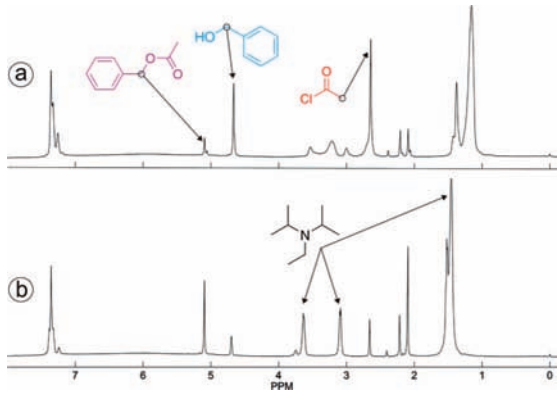


***Figure 3.*** In situ NMR monitoring of the acetylation of benzyl alcohol in the presence of DIPEA. Spectra at reaction times of (a) 9 s and (b) 3 min are shown. Stoichiometry: benzyl alcohol/DIPEA/acetyl chloride = 1:1:1.2.

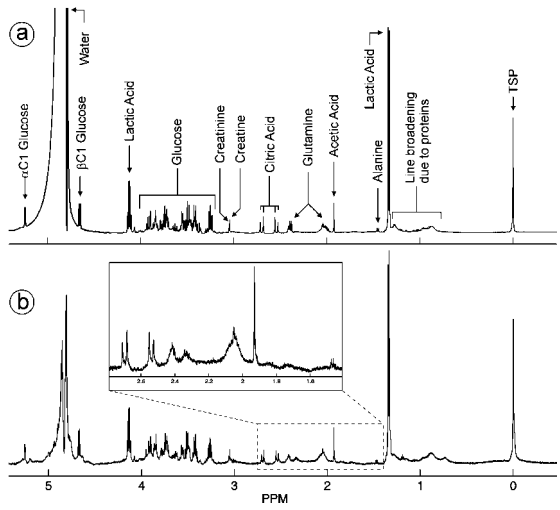


***Figure 4.*** NMR spectra of human CSF as recorded in (a) a 5 mm probe and (b) the microchip, which contained 450 $\mu$L and 600 nL, respectively. Conditions: (5 mm probe) 256 scans, 90° pulse width (PW) of 6 $\mu$s, pH 9.89; (microchip probe) 4608 scans, 90° PW of 7 $\mu$s, pH 9.41.

Because of the inherently high resolution, line broadenings can be interpreted as meaningful information about the sample. In the current experiments, peak broadenings and shifts of the DIPEA resonances were observed and attributed to partial protonation of DIPEA at short reaction times (<15 min). Moreover, the microreactor experiments showed a resonance at 2.40 ppm that was not observed in experiments performed in a 5 mm NMR tube. These results demonstrate the possibility of tracking intermediates using fast in situ analysis. An in-depth study of this reaction is underway.

Although it was not specifically built for this purpose, we tested the microfluidic stripline resonator to establish its suitability for metabolomics studies. Figure 4 shows $^1$H NMR spectra of human cerebrospinal fluid (CSF) as measured in a conventional 5 mm probe for reference and in the stripline chip. The composition of CSF, which surrounds the spine and brain, reflects the cytological and biochemical basis of central nervous system diseases, inborn metabolic errors, and metabolism of different diseases.[11] A 5 $\mu$L sample prepared from the reference CSF sample (concentrated 9 times in $D_2O$; see the Supporting Information) was injected into the chip with a 600 nL detectable volume, thus containing 1.2% of the metabolites in the reference sample. In both 1D spectra, all of the main metabolite resonances in the aliphatic region could be identified successfully. Small chemical-shift changes between the two spectra are due to pH differences. The stripline spectrum is slightly broader, as a lock channel has yet to be implemented. The lowest intensity peak that could be identified was for alanine (1.45 ppm). The concentration of this metabolite was 1.21 mM, corresponding to 0.7 nmol in 600 nL. The sensitivity enhancement of the stripline probe relative to the reference probe was a factor of 3.4. This enhancement is rather moderate considering the sensitivity reported previously for the stripline. However, the sensitivity can be improved considerably by the use of substrates with lower losses and optimization of the filling factor. Furthermore, an optimized stripline for metabolomics studies should have an appropriate volume that takes into account both the overall amount of material available and the solubility of the various compounds. Finally, preparatory sample handling should preferably be integrated into the microfluidic device along with methods to maximize the "observe factor", which could be achieved by introducing plug flow. Currently we are contemplating such a design. On the basis of the experience with our prototype stripline probe,[8a] we anticipate measurement times of a few minutes for the analysis of mouse CSF, where ~7 $\mu$L is available ante mortem.[12]

In conclusion, we have demonstrated an integrated microfluidic NMR flow probe for the study of reaction kinetics. Furthermore, we have demonstrated the possibility of using the stripline design for screening of mass-limited biological samples. With the microfabrication toolbox available nowadays, a completely integrated platform in one chip that can handle and detect raw samples without preparative laboratory work is within reach.

**Acknowledgment.** We thank J. van Os for fruitful discussions and R.-J. Lamers (Erasmus University Rotterdam) for providing the CSF sample. A.J.K., K.A.M.A., and S.S.W. acknowledge financial support from Top Institute Pharma (Project D4-102). This project was funded by the Dutch NWO ACTS Process on a Chip Program.

**Supporting Information Available:** Experimental details, sensitivity calculations, a Lorentzian fit of the methyl peak of ethanol, and the reference spectrum of the acetylation reaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA900389X