IN-LINE NMR ANALYSIS USING STRIPLINE BASED DETECTORS

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ABSTRACT

This contribution describes a silicon-based microfluidic chip with an integrated rf-stripline for NMR detection, with high spectral resolution (0.7 Hz at 600 MHz proton resonance), with a particular focus on in-line chemical reaction monitoring. The timescale for kinetic monitoring can be brought down to the millisecond range.

KEYWORDS: NMR, stripline, microfluidic, reaction monitoring

INTRODUCTION

Nuclear magnetic resonance (NMR) is a well-established spectroscopic technique for the identification of chemical species and is broadly applied in many different fields like synthetic and supramolecular chemistry, catalysis, materials science, biology and medicine. In a simple NMR experiment, the sample is exposed to a static magnetic (B_0) field. After excitation of the nuclear spin system using a short radio frequency (rf) puls, the precessing magnetization is detected. The recorded resonance frequencies (peaks in the NMR spectra) are a probe of the local electronic environment of a specific nucleus in a molecule. Additionally, fine structure like J-couplings and dipolar couplings are a measure of chemical bonding and distance between two nuclei, respectively. NMR is a non-invasive technique and provides direct quantitative information.

Recent developments have shown a focus on reducing the NMR detection volume to make it compatible with microliter and even nanoliter volumes of analytical separation techniques like Liquid Chromatography (LC) and Capillary Electrophoresis (CE) [1]. Furthermore, microcoil NMR is used as a method to follow reaction kinetics [2]. However, due to its intrinsically low sensitivity, these in-flow monitoring experiments remain to be a challenge. Traditionally, NMR on small volumes is performed by means of small solenoids wrapped around a capillary [3], or planar coils on glass chips containing microfluidic channels [2,4,5]. The most important problem of these approaches is the fact that the nearby copper windings of the rf-coil induce static field distortions that limit the resolution and (indirectly) the Signal to Noise (SNR) performance.

To overcome these problems, we have developed an NMR detector based on a conceptually different element, the so-called rf-stripline[6]. We have implemented this new type of NMR detector in a microfluidic chip [7,8]. A microreactor chip was connected to the NMR chip, so that chemical reactions can be monitored. In this contribution we show the performance of stripline-based NMR detectors for the in-line analysis of a model reaction. We show a modification of the stripline geometry which allows one-dimensional imaging and fast reaction kinetic monitoring down to the ms regime.

STRIPLINE DESIGN AND FABRICATION

The stripline design consists of a flat strip confined between two metal shielding planes, which in microwave electronics usually is called a stripline configuration (Figure 1). To obtain a high rf-field strength, which is necessary for high sensitivity, the strip is constricted in the center as is shown in Figure 1a-c. Due to this constriction, the current density increases locally, and with that the rf-field strength. The microstrip has highest relative volume with a B_1 deviation of less than 10%, i.e. the highest effective filling factor and thus highest sensitivity. Because of the symmetric copper geometry and the in-line positioning of the stripline parallel to the B_0-field, in comparison to solenoid of flat helical coils, minimal static field distortions are induced ensuring high spectral resolution.

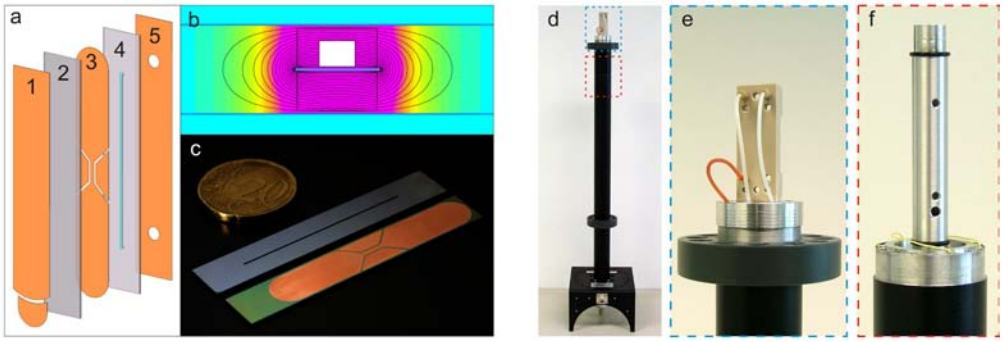

Figure 1: (a) Exploded view of the microfluidic NMR chip. Layers 1, 5: grounded copper layers, layers 2,4: low-loss silicon platelets; layer 4 contains a microfluidic channel; Layer 3: copper stripline. (b) Cross-section of stripline chip, showing magnetic field lines. White rectangle: microfluidic channel, outlined box: area of uniform magnetic field. (c) Photograph of microfabricated chip parts before bonding. (d) The custom-made microfluidic probe. The dashed line indicates the position of the NMR chip. (e) Close-up view of the microreactor holder mounted on top of the probe. (f) Close-up view of the stripline chip holder.

Fabrication of the stripline chips comprises Deep Reactive Ion Etching of a microchannel in a high-resistivity silicon substrate and depositing a copper ground plane on its backside, electroplating the stripline structure on a second high-resistivity silicon substrate, and depositing again a copper ground plane on its backside, and finally bonding the two substrates together. The detected sample is 600 nL under the constriction. The chip is mounted in a vertical bore probe, which contains the fluidic and electrical connections, see Figure 1d-1f.

RESULTS AND DISCUSSION

Single shot experiments showed unsurpassed resolution. Initial measurements with a non-integrated system containing only 12 nL sample have been carried out succesfully [6]. In our fully integrated microfluidic chip containing 600 nL of pure ethanol, a SNR of 5500 and a linewidth of 0.0012 ppm (0.7 Hz) full-width-at-half-maximum (FWHM) were achieved. Typical J-multiplets were resolved down to the baseline. This is the highest resolution ever achieved on chip, and competitive with standardized NMR-equipment.

For the reaction monitoring, a Micronit microreactor chip (Y-junction type) was implemented on top of the probe as shown in Figure 1d and 1e. Two syringe pumps are used to control the flow of the reactants, capillaries are connecting the syringes to the inlets of the microreactor. The reaction starts in the 142 nL volume reaction channel of the reaction channel and subsequently the products flow through the stripline where the detection takes place. The total reaction volume is 4.5 μL. By changing the flow rate, the reaction time can be varied from several seconds up to 10 minutes.

We studied the first minutes of the acetylation of benzyl alcohol in the presence of DIPEA. Acetyl chloride (1.2 M in $CDCl_3$) was combined with benzyl alcohol (0.5 M) and DIPEA (n,n-diisopropylethylamine, 0.5M) in $CDCl_3$. Figure 2a shows spectra at different reaction times. One can observe the alpha protons of benzyl acetate (5.2 ppm, marked in green) and benzyl alcohol (4.7 ppm, marked in blue), acetyl chloride (2.7 ppm), an intermediate (2.4 ppm), acetic anhydride (2.25 ppm), benzyl acetate (2.15 ppm), the three DIPEA peaks that shift and split during the reaction, in orange/red and the benzyl aromates (7.3 ppm). The conversion of the reaction, that can be monitored from the ratio of the peaks of benzyl alcohol and benzyl acetate, goes up to 70% during the first ten minutes. The shifting and splitting of the DIPEA peaks is found to arise from protonation in the first part of the reaction and DIPEA-acetate complex formation in the later part of the reaction.

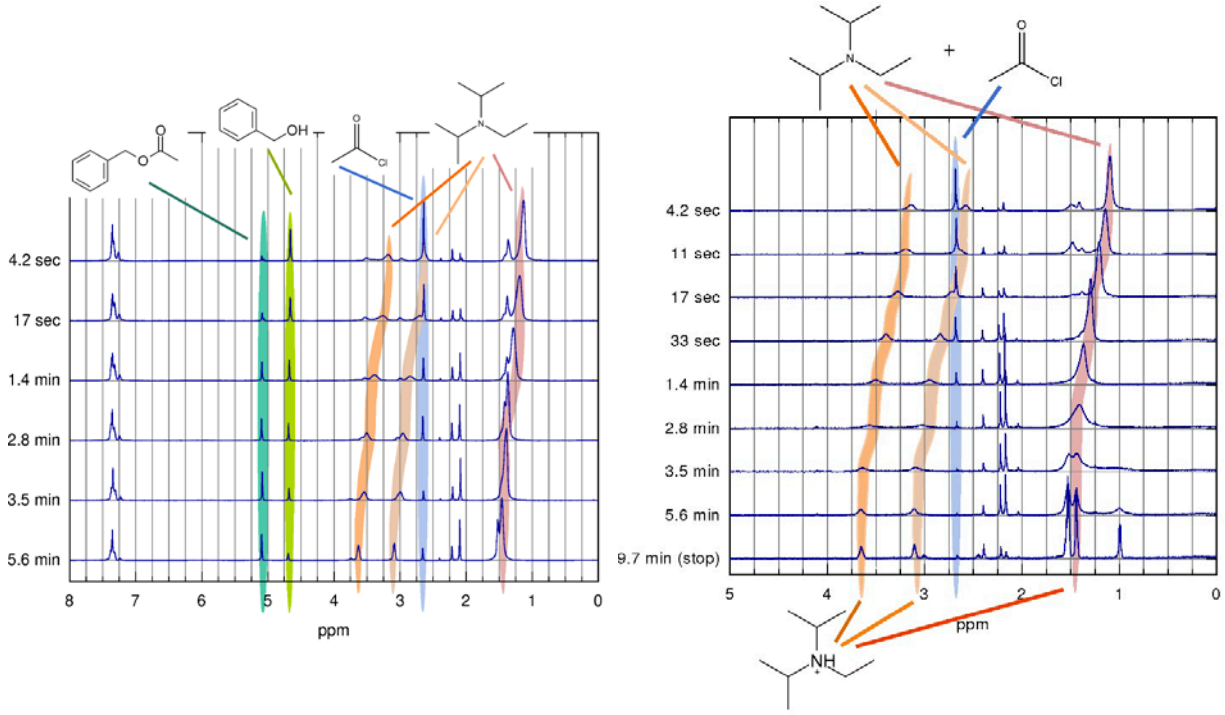

Figure 2: (a) Spectra of the acetylation of benzyl alcohol in the presence of DIPEA, reaction time from 4.2 seconds to 5.6 minutes. (b) Spectra of the reaction of DIPEA with acetyl chloride, reaction time from 4.2 seconds to 9.7 minutes.

We found that the kinetics of the conversion does not agree with a second order reaction mechanism, where DIPEA is only functioning as a proton scavenger. Secondly, the presence of an intermediate compound and the shifting and splitting of the DIPEA peaks suggest a more complex reaction mechanism where DIPEA is actively involved in the reaction. Therefore, the reaction of acetyl chloride with DIPEA is studied in more detail. A series of spectra has been measured in the stripline following the reaction in the micro reactor (AcCl and DIPEA: 0.5 M in $CDCl_3$), shown in Figure 2b. As in the case with benzyl alcohol, the acetyl chloride peak (2.7 ppm) decreases and the DIPEA peaks split and shift in the course of the reaction. Also, acetic anhydride is present at 2.25 ppm and two intermediate products are found at 2.18 and 2.4 ppm. From this, we conclude that acetyl chloride reacts with DIPEA by forming protonated DIPEA and ketene. This is confirmed by trace amounts of diketene, found in regular liquid NMR measurements. The partial protonation of DIPEA accounts for the shifting and broadening of the DIPEA resonances. Further splitting of the DIPEA peaks in a later stage of the reaction and the development of extra lines suggest that protonated DIPEA reacts further into a DIPEA-

acetate complex. Considering the acetylation of benzyl alcohol and these findings, a reaction mechanism is proposed where benzyl alcohol reacts with ketene and/or the DIPEA-acetate complex into benzyl acetate. More research is presently ongoing to solve the details of the reaction mechanism.

The stripline probe has shown its potential for high-resolution monitoring of chemical reactions. The substrate, however, turned out to be non-optimal in terms of sensitivity, inducing substantial rf-losses. Therefore, we are currently fabricating chips from fused silica substrates which are expected to provide significantly better sensitivity. Furthermore, the sensitivity is straightforwardly enhanced by fabrication of two fluidic channels at both sides of the central strip. To extend the applicability of this new probe, a ^{13}C rf-channel has been implemented.

An interesting property of striplines is that varying the local width makes it possible to create an rf-field profile, making it possible to use striplines for imaging (MRI) or very fast kinetic purposes. Mostly, MRI is performed using gradients in the external magnetic field to code for position through the local resonance frequency of the spins. Alternatively well-defined gradients in the rf-field strengths can be used to encode for position through the local nutation frequency of the spins in the rotating frame. We have developed a stripline-based MRI probe, with a tapered constriction instead of a straight one, resulting in a linear gradient in rf-strength. This rf-gradient allows for encoding in one spatial dimension, but the probe simultaneously provides chemical shift information. We are currently exploring this concept for the monitoring of very fast chemical reactions. For this, two flowing reactants are mixed in a capillary above the stripline such that the reaction starts at the front of the constriction and ends at the back of the constriction. Under (constant) flow different positions above the stripline therefore correspond to different reaction times that can be mapped out by the rf-gradient. The reaction mixture now moves over millimeters during the reaction bringing the minimum detectable reaction time down to the milliseconds regime as compared to seconds in the previous approach which was due to the transport time from the reactor to the NMR-chip. How far down one can push the reaction time depends on the necessary resolution as flow rates which are faster than the data acquisition time, will bring down spectral resolution. Initial tests monitoring a simple reaction of diethylamine and formic acid show encouraging results.

CONCLUSION

Striplines are a promising alternative to solenoid and planar coils for NMR detection of volume restricted samples. We have demonstrated in-line NMR monitoring of the acetylation of benzyl alcohol and are investigating the reaction pathway in detail. Sensitivity of the stripline resonators is competitive but leave room for further improvements by an appropriate choice of substrates. Miniaturized NMR detection also opens a route toward efficient implementation of nuclear hyperpolarization schemes such as Dynamic Nuclear Polarization (DNP) with potential signal gain of hundreds [9]. Due to versatility of the stripline geometry it can be scaled to the desired dimension and allows for in traducing well-defined rf gradients which can be used for 1D imaging without loss of spectral information. This holds great promises for the monitoring of fast reactions kinetics down to the millisecond regime.

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