Continuous-Flow NMR Spectroscopy

Measuring Reaction Kinetics by Using Multiple Microcoil NMR Spectroscopy**

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Knowledge of kinetics is an important component in investigating reaction mechanisms. Such information greatly benefits drug design,[1] drug metabolism,[2] and a variety of industrial processes.[3] A number of different analytical techniques can be employed to investigate kinetic data. Spectroscopic tools are particularly useful because, besides

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[**] This work was supported by the U.S.A. NIH (8R01EB002343-07), the NSF and the Alexander von Humboldt Stiftung (AGW).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200351901

reaction rates, they can provide chemical information at the molecular level. Nuclear magnetic resonance (NMR) is one of the most information-rich techniques, able to provide a high degree of structural information. However, poor sensitivity and subsequent long measurement times often preclude the acquisition of real-time NMR data for chemical reactions involving small amounts of material. A simple and effective approach to improve the mass sensitivity of NMR is to employ solenoidal NMR microcoil detectors. It has been shown both theoretically and experimentally that reducing the diameter of the coil increases its sensitivity,[4] thus allowing the analysis of pL to μL sample volumes. Since the introduction of high-resolution microcoil NMR probes[5] a number of applications have been demonstrated. NMR microcoils have been successfully used as online detectors in capillary separation[6] and for protein-unfolding kinetics.[7]

NMR has been used for the direct observation of short-lived species in chemical reactions by using rapid injection[8] and continuous-flow techniques.[9] In the present study we combine the technique of continuous reactant flow with multiple microcoil NMR detection in a new and efficient approach to obtain kinetic information on reactions that take place on timescales between seconds and minutes. As a model system we have studied xylose–borate reaction kinetics.

Two fluid flows that contain the reactants are mixed and this mixture passes through a capillary around which are wound multiple, physically distinct NMR detector coils. The distance between the mixer and each individual NMR coil, together with the flow rate used, determines the post-reaction-time point. Signal averaging can be performed for as long as necessary to obtain an adequate signal-to-noise ratio, as the time of the data measurement and the reaction time are decoupled through this experimental method. However, the longer the required total data-acquisition time, the greater the amount of reactants are needed. The use of multiple small-volume microcoils, three in this case, (Figure 1), minimizes this amount.

The ^{1}H NMR spectra of D-xylose (300 mM) and the equilibrium mixture D-xylose plus borate (400 mM, pD 10) solutions are shown in Figure 2 a and b, respectively, with no flow present. The NMR line broadens as the flow rate increases[10] and also its intrinsic shape changes. With no flow the lineshape was measured to be a pure Lorentzian with a

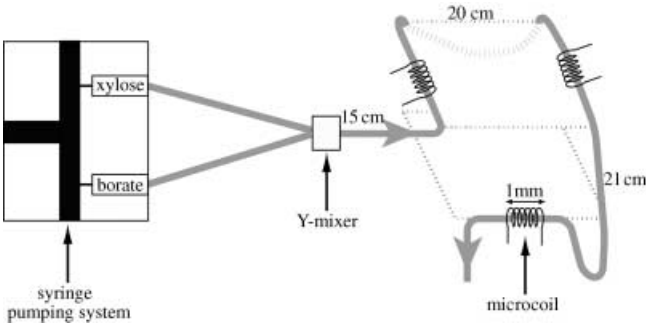


Figure 1. Schematic of the experimental setup. Two syringes on the pump inject the reactants into two capillaries. The reactants are mixed rapidly at the Y-mixer. After mixing, the solution flows through the microcoils while data are being acquired.

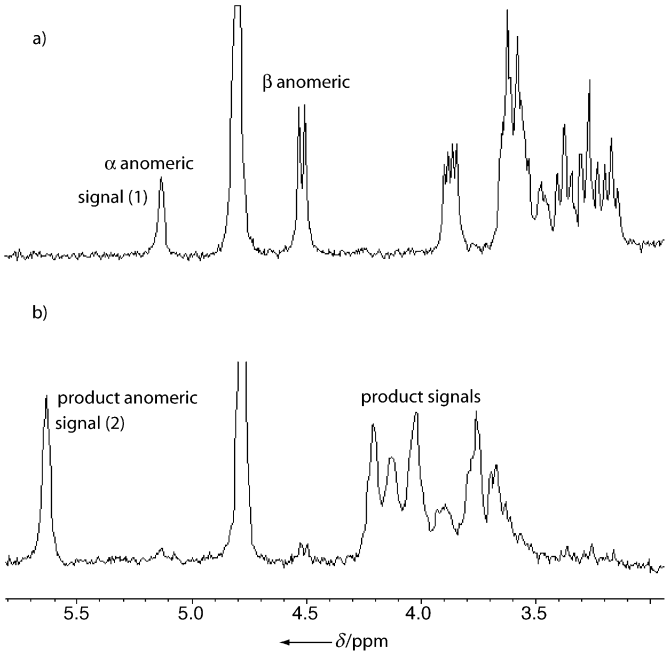
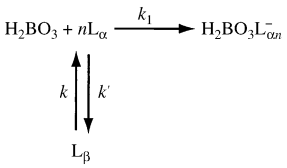


Figure 2. ^{1}H NMR spectra of 300 mM a) D-xylose in D_2O and b) D-xylose plus 400 mM borate at pD 10. The spectrum in b) corresponds to the equilibrated product mixture. Chemical shifts are referenced to HDO at 4.78 ppm.

full-width-half-maximum (FWHM) of 2.5 Hz. As the flow rate was increased to 18 μL min^{-1}, the nominal linewidth increases to ≈6 Hz, with a lineshape described by a Voigt function, defined by the convolution of Gaussian and Lorentzian functions.[11] The fractional Lorentzian linewidth was 44 % and Gaussian linewidth 56 %. Given the substantial alterations in NMR lineshape with flow, the most robust method of data analysis is to measure the ratio of signal areas of product and reactants by using specific resonances as shown in Figure 2. The use of these ratios also enables us to combine data acquired from the three different radio-frequency (RF) coils, which may have slightly different line widths and sensitivities.

The formation of stable borate–sugar complexes has been the subject of numerous studies.[12] The reaction kinetics model for the D-xylose-borate reaction is represented in Scheme 1, in which L_α and L_β represent the α and β forms of D-xylose, $H_2BO_3^-$ the borate ion and $H_2BO_3L_{\alpha n}^-$ the 1:*n* borate–xylose complexes. The NMR signals shown in Figure 2 b correspond to the reaction product under equilibrium conditions. Weak background signals arise from D-xylose. "On-flow" time resolved or stopped-flow NMR measurements do not provide evidence for significant back reaction.



Scheme 1. The reaction kinetics model for the D-xylose-borate reaction.

Therefore, it can be safely assumed the D-xylose–borate reaction is effectively irreversible at the pD value of 10 used in these experiments. In the presence of borate, the conversion of L_β to L_α is fast, and one can therefore assume that $L_\alpha = \text{const}\, L_{total}$. Experiments were performed in which the initial borate concentration was higher than the D-xylose concentration, which allowed the consideration of only 1:1 complexes ($H_2BO_3L^-_{\alpha n}$). Thus, the reaction is described by the following set of differential equations (based on the assumption of a second-order reaction) [Eq. (1)]:

$$\begin{cases} -\frac{d[L_\alpha]}{dt} = k_1[B^-][L_\alpha] \\ -\frac{d[B^-]}{dt} = k_1[B^-][L_\alpha] \\ \frac{d[BL^-_\alpha]}{dt} = k_1[B^-][L_\alpha] \end{cases} \quad (1)$$

By solving the system above the time dependence of the ratio $[H_2BO_3L^-_{\alpha n}]/[L_\alpha]$ is found to be [Eq. (2)]:

$$\frac{[H_2BO_3L^-_{\alpha n}]}{L_\alpha} = \frac{m_2 + m_3 \exp(m_1 k_1 t)}{m_1} \quad (2)$$

in which the reaction rate constant k_1 and the coefficients m_1, m_2 and m_3 are to be estimated from the experimental data.

As the reaction progresses, a decrease in the height of the α and β anomeric peaks and the appearance of a new peak (denoted product anomeric in Figure 2b), at 5.55 ppm, were observed. NMR spectra that correspond to successive reaction times were recorded independently and simultaneously with all three RF coils. The results from a quantitative analysis of the spectra are presented in Figure 3. The data from all three probes are plotted on the same axis in this figure and fall on the same curve, thus validating the use of distinct NMR coils at multiple locations along the capillary/reaction-time coordinate. The ratio of the signal heights, height of signal (2)/height of signal (1) (Figure 2) is plotted as a function of time. By fitting the experimental data to the theoretical $[BL^-_{\alpha n}]/[L_\alpha]$ ratio given by Equation (2), a reaction rate constant, $k_1 = 0.077 \pm 0.004\ s^{-1}\ mol^{-1}\ L$, for the second order reaction was obtained.

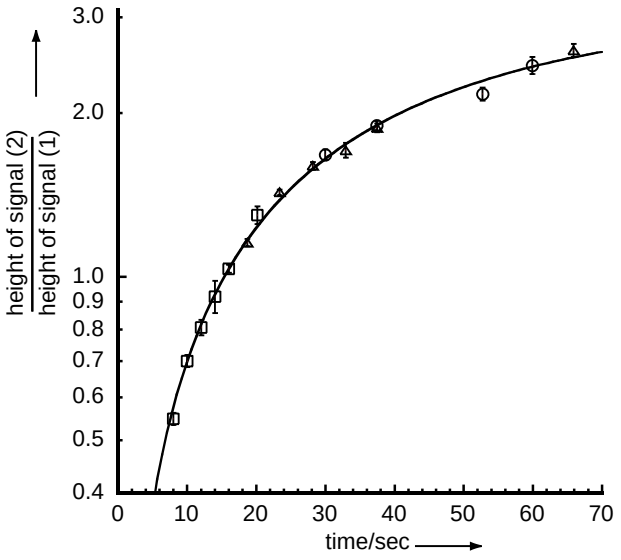


Figure 3. The ratio of the signal amplitudes, height of signal (2)/height of signal (1), as a function of time. □ coil 1; △ coil 2; ○ coil 3. The error bars represent the standard error from ten measurements. The fit parameters, Equation (2), are: $m_1 = -0.316$, $m_2 = -1$ and $m_3 = 0.994$.

In summary, this work demonstrates the possibility of using NMR as an analytical tool to study chemical kinetics of small amounts of materials with reaction times of a few seconds or longer by using continuous-flow NMR with multiple microreceiver coil detection. Continuous flow decouples the total data-measurement time from the reaction time, and the use of multiple microcoils decreases the amount of sample required to obtain the data. Highly reproducible results from a xylose–borate system, which show excellent agreement with the predicted mathematical model, validate this approach and demonstrate its utility for obtaining kinetic information on chemical species difficult to measure using other detection modalities. Moreover, the method can also be used to perform continuous-flow 2D NMR experiments at a particular reaction time,[13] which demonstrates the ability to examine intermediate species. A further expansion of the probehead to include four to six coils appears possible, with a concomitant reduction in the total amount of material needed for a kinetic study.

Experimental Section

Experiments were carried out at 300 MHz on a wide bore (89 mm) magnet by using a four-receiver channel Apollo Tecmag console. RF pulses were transmitted from a single frequency source through a power-splitter to each coil. The NMR probe consisted of three identical 370 μm diameter solenoidal RF microcoils, constructed of 50 μm copper wire wrapped around polyimide tubing.[14] Each coil was about 1 mm long and had seventeen turns. The sample flowed through a 200 μm internal diameter capillary, which resulted in a detection volume of ≈31 nL. The three-coil-probe assembly was immersed in FC-43 to minimize susceptibility-mismatch distortions.[15] FWHM linewidths of 1–2 Hz were obtained for HDO (2% H_2O in D_2O) for single coils. Simultaneous data acquisition performed by using optimized shimming for all three coils yielded linewidths of 4–6 Hz.

Interactions between different coils were measured both electrically and by NMR. Table 1 lists the S_{21} parameters for the three-coil configuration, while Table 2 contains the NMR measurements of

Table 1: S_{21} parameter (in dB) for intercoil coupling.

Coil	1	2	3
1	–	−40	−34
2	−40	–	−45
3	−34	−45	–

Table 2: NMR signal bleedthrough between coils measured using the S/N of the HDO peak.

Coil	1	2	3
1	–	0.0035	0.0050
2	0.0035	–	0.0022
3	0.0050	0.0022	–

signal cross-contamination. The S_{21} parameter, with suitably impedance-matched loads, measures the ratio of the output voltage across one coil when an input voltage is applied to a second coil; for zero intercoil coupling this value should be $-\infty$ dB. All the microcoils gave values between -30 and -50 dB, thus indicating extremely high isolation. To measure the NMR intercoil signal bleedthrough the system was configured to transmit on only one coil and receive on all three coils. A total of 100 scans were acquired with a relaxation delay of 750 ms, and the signal to noise (S/N) of the HDO signal was measured for each coil. The numbers displayed in Table 2 were obtained by dividing the S/N values for each coil pair. The results show a very small degree of cross talk in agreement with the electrical measurements.

D-xylose and borate solutions were mixed rapidly by using a Y-mixer. The mixed solution flowed continuously from the Y-mixer through all three RF coils. The reaction times at which the measurements were made were defined by the physical separation of the detection coils from the micromixer and the flow rate used. The distances between the three detection points are shown in Figure 1. Flow rates ranging from 17.6 $\mu L\,min^{-1}$ to 5 $\mu L\,min^{-1}$, which correspond to reaction times between 8 s and 66 s, respectively, were used (as there are two capillaries entering the Y-mixer, a flow rate f delivered by the syringe pump corresponds to an effective flow rate $2f$ in the capillary running through the NMR coils).

Received: May 12, 2003
Revised: July 4, 2003 [Z51901]

Keywords: analytical methods · kinetics · NMR spectroscopy · reaction mechanisms

[1] R. Almog, C. A. Waddling, F. Maley, G. F. Maley, P. Van Roey, *Protein Sci.* **2001**, *10*, 988.
[2] J. M. Hutzler, T. S. Tracy, *Drug Metab. Dispos.* **2002**, *30*, 355.
[3] X. S. Chai, Q. Luo, J. Y. Zhu, *J. Chromatogr. A* **2002**, *946*, 177.
[4] A. Abragam, *The Principles of Nuclear Magnetism*, Clarendon Press, Oxford, **1961**.
[5] D. L. Olson, T. L. Peck, A. G. Webb, R. L. Magin, J. V. Sweedler, *Science* **1995**, *270*, 1967.
[6] M. E. Lacey, Z. J. Tan, A. G. Webb, J. V. Sweedler, *J. Chromatogr. A* **2001**, *922*, 139; M. E. Lacey, A. G. Webb, J. V. Sweedler, *Anal. Chem.* **2000**, *72*, 4991; A. M. Wolters, D. A. Jayawickrama, C. K. Larive, J. V. Sweedler, *Anal. Chem.* **2002**, *74*, 4191.
[7] M. Kakuta, D. A. Jayawickrama, A. M. Wolters, A. Manz, J. V. Sweedler, *Anal. Chem.* **2003**, *75*, 956.
[8] J. F. McGarrity, C. A. Ogle, Z. Brich, H.-R. Loosli, *J. Am. Chem. Soc.* **1985**, *107*, 1810.
[9] C. A. Fyfe, S. W. H. Damji, A. Koll, *J. Am. Chem. Soc.* **1979**, *101*, 951; C. A. Fyfe, M. Cocivera, S. W. H. Damji, *J. Am. Chem. Soc.* **1975**, *97*, 5707; K. Albert, E.-L. Dreher, H. Straub, A. Rieker, *Magn. Reson. Chem.* **1987**, *25*, 919.
[10] *On-line LC-NMR and Related Techniques* (Ed.: K. Albert), Wiley, West Sussex, **2002**.
[11] T. Ida, M. Ando, H. Toraya, *J. Appl. Crystallogr.* **2000**, *33*, 1311.
[12] S. Chapelle, J. F. Verchere, *Tetrahedron* **1988**, *44*, 4469; J. F. Verchere, M. Hlaibi, *Polyhedron* **1987**, *6*, 1415.
[13] For continuous-flow 2D NMR experiments see Supporting Information.
[14] R. A. Kautz, M. E. Lacey, A. M. Wolters, F. Foret, A. G. Webb, B. L. Karger, J. V. Sweedler, *J. Am. Chem. Soc.* **2001**, *123*, 3159.
[15] Y. Li, A. M. Wolters, P. V. Malawey, J. V. Sweedler, A. G. Webb, *Anal. Chem.* **1999**, *71*, 4815.

