COMMUNICATION

Single-shot titrations and reaction monitoring by slice-selective NMR spectroscopy†

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A new method, based on slice-selective NMR spectroscopy of inhomogeneous mixtures, is introduced to perform NMR titrations and reaction monitoring in a single experiment. The method was applied to the titration of a lithium salt with 12-crown-4, and to the reaction of *n*BuLi with *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDTA).

NMR spectroscopy plays an increasingly important role in the elucidation of structural and dynamic features of inorganic, organic and biomolecular compounds and their interactions. For example, chemical shift titration is a powerful method to determine the stoichiometry and stability constants of complexes in coordination, supramolecular and medicinal chemistry.[1–3] In such an experiment, the chemical shift of a particular resonance attributed to one component (*e.g.* the metal, host or target) is monitored in a series of NMR spectra, while the concentration of the other component (*e.g.* the guest or ligand) is systematically varied. In a different approach, NMR is applied to investigate chemical reactions by monitoring resonances of substrates and products in a series of individual NMR spectra over time. The observation of fast reactions and short-lived intermediates[4,5] has become possible through custom build NMR hardware and techniques such as stopped flow[6,7] and rapid-injection NMR.[8–12]

In our research group slice-selective NMR spectroscopy has lately become an important technique to study diffusion of solvents and solutes into polymers.[13] Using standard solution NMR instrumentation, slice-selection is accomplished by shaped radio-frequency pulses with variable frequency offsets in the presence of a magnetic field gradient along the axis of the NMR tube.[13–17] For highly sensitive nuclei such as ^1H, ^7Li, ^{19}F or ^{31}P, a series of typically ∼20 conventional NMR spectra of individual horizontal (∼1 mm) slices within the active sample volume (∼2 cm) is obtained in less than 2 min. Here we propose a fast chemical shift

titration method based on slice-selective NMR spectroscopy in combination with a concentration gradient of the ligand component rather than incrementing the concentration step-by-step. Alternatively, chemical reactions between two substrates diffusing towards each other may be monitored.

As a case study for the slice-selective titration method we investigated the complexation of a ^7Li ion with 12-crown-4. For this purpose, 12-crown-4 (50 μL, 3.1 mmol, m.p. 16 °C) was filled into a standard 5 mm NMR tube and cooled to 5 °C. Then a solution of LiClO$_4$ (24 mg, 2.3 mmol) in acetonitrile-d_3 (0.45 mL) was layered on top of the solid ether. This procedure prevents initial mixing of both components prior to the measurements. Inside the NMR magnet (25 °C, standing tube) the ether melts and slowly diffuses into the LiClO$_4$ solution resulting in a smooth concentration gradient along the tube axis. Diffusion of LiClO$_4$ into the ether phase occurs likewise, but with little impact on the ^7Li concentration due to the 9 : 1 volume ratio. Likewise, the impact of molecular diffusion during the gradient pulse is negligible. Approximately 3, 6 and 9 h after sample preparation slice-selective ^1H and ^7Li NMR measurements were performed (see ref. 13 and ESI† for details).[13] For each slice, absolute integrals of both, the ^7Li resonance of LiClO$_4$ and the ^1H resonance of 12-crown-4, were measured and converted into concentrations using homogenous reference samples. This way, the ^7Li chemical shift observed in each slice can be assigned to a specific ether/lithium ratio.

Fig. 1 shows the series of 19 slice-selective ^7Li NMR spectra recorded after 6 h (for spectra recorded after 3 and 9 h see ESI†). Additional slice-selective ^1H spectra (see also ESI†) confirm that 12-crown-4 diffuses from the bottom to the top and builds up a smooth gradient, while the LiClO$_4$ concentration is approximately constant. Slice 1 (at the top of the active volume) shows a narrow ^7Li resonance at −2.3 ppm, typical for Li$^+$ coordinated by four acetonitrile molecules. As the ether concentration increases, the ^7Li resonance is shifted downfield and reaches a maximum (−0.8 ppm) in slice 12, where the 12-crown-4 and LiClO$_4$ concentrations are approximately equal and thus the [Li(12-crown-4)]$^+$ complex dominates.[18] In the presence of an excess of 12-crown-4, the ^7Li resonance is then shifted again upfield until it reaches −1.2 ppm

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† Electronic supplementary information (ESI) available: Detailed procedures, spectra, additional schemes. See DOI: 10.1039/c4cc08329f
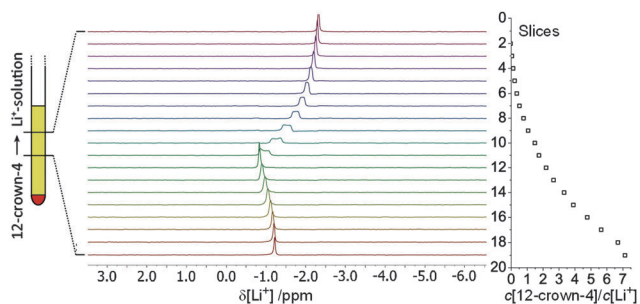
Fig. 1 Slice-selective ^{7}Li NMR spectra of LiClO$_4$ in acetonitrile-d_3 in the presence of a concentration gradient of 12-crown-4, 6 h after sample preparation. Generally, slices (1 mm thick, 1 mm distance each) are numbered from the top of the active volume to the bottom, with slice 10 located at the centre. The 12-crown-4/LiClO$_4$ concentration ratio is indicated on the right and increases from slice 1 to slice 19.

in slice 19 (7-fold excess, [Li(12-crown-4)$_2$]$^+$). The resulting titration curve (see ESI†) fully agrees with previous reports. Note that the ^{7}Li resonances in slices 6 to 11 are increasingly broadened due to (rectangular weighted) summation over the ether concentration gradient within each slice. This broadening could be overcome by further decreasing the slice width, thereby increasing the spatial resolution along the tube axis, but at the cost of reduced sensitivity, which may render weaker signals undetectable.[18,19]

The application of slice-selective NMR spectroscopy to reaction monitoring is demonstrated with the reaction between nBuLi and PMDTA. Due to the high reactivity of organolithium reagents and to prevent rapid convection and/or diffusion out of the active sample volume, nBuLi was first diffused into a polystyrene gel matrix, and all steps of the preparation were carried out strictly under argon atmosphere. For this purpose, a solution of nBuLi in n-hexane (0.4 mL, 1.9 M, 0.74 mmol) was concentrated *in vacuo* and toluene-d_8 (0.40 mL) was added. This solution was then

transferred into a 5 mm NMR tube, and a cylindrical polystyrene stick (10 × 3.8 mm, crosslinked through 0.2 vol% divinylbenzene, for preparation see ref. 20) was immersed in the solution approximately 1 cm above the bottom of the tube. After 7 days (the polystyrene stick had swollen to a length of ∼3 cm) the supernatant nBuLi–toluene solution above the polymer was removed and replaced by a solution of PMDTA (0.12 mL, 0.60 mmol) in toluene-d_8 (0.15 mL). Slice-selective ^{1}H and ^{7}Li NMR measurements started after ∼3 h and went on for three days.

Fig. 2 (bottom left) shows the slice-selective ^{7}Li NMR spectra three days after the addition of PMDTA to the polymer imbibed with nBuLi (for spectra recorded after 3 h, 1 and 2 days, see ESI†). Slices 19 and 18 at the bottom of the active volume still show a signal for unreacted nBuLi at 2.3 ppm, in agreement with the absence of ^{1}H signals of PMDTA in these slices (see also ESI†). On the other hand, in slices 1–12 a narrow resonance at 1.1 ppm is observed, which was assigned to the product of the reaction, lithiated PMDTA (see Fig. 2 top). Satellite peaks in slices 6–12 arise from residual quadrupolar couplings (RQCs) due to partial orientation of molecules inside the stretched gel.[20] The size of the RQCs varies with the amount of strain and is not constant over the gel body unless the gel is equilibrated for a prolonged period of time.[13,15] Slices 1 to 5 are located outside the polymer and hence only a singlet is observed for lithiated PMDTA in this region.

Increasingly broad signals in slices 17–13 with chemical shifts between 2.3 and 1.6 ppm mark the reaction front between PMDTA and nBuLi, at which multiple dynamic processes take place.[21] Although the corresponding ^{1}H spectra are relatively crowded with signals of different reaction components as well as residual signals from the polymer, the isolated region of the nBuLi α-CH$_2$ protons (−0.5 to −1 ppm) is quite informative (Fig. 2 bottom right): the signal for the nBuLi hexamer[22,23] at −0.92 ppm moves downfield and broadens from slice 19 to 13, presumably due to
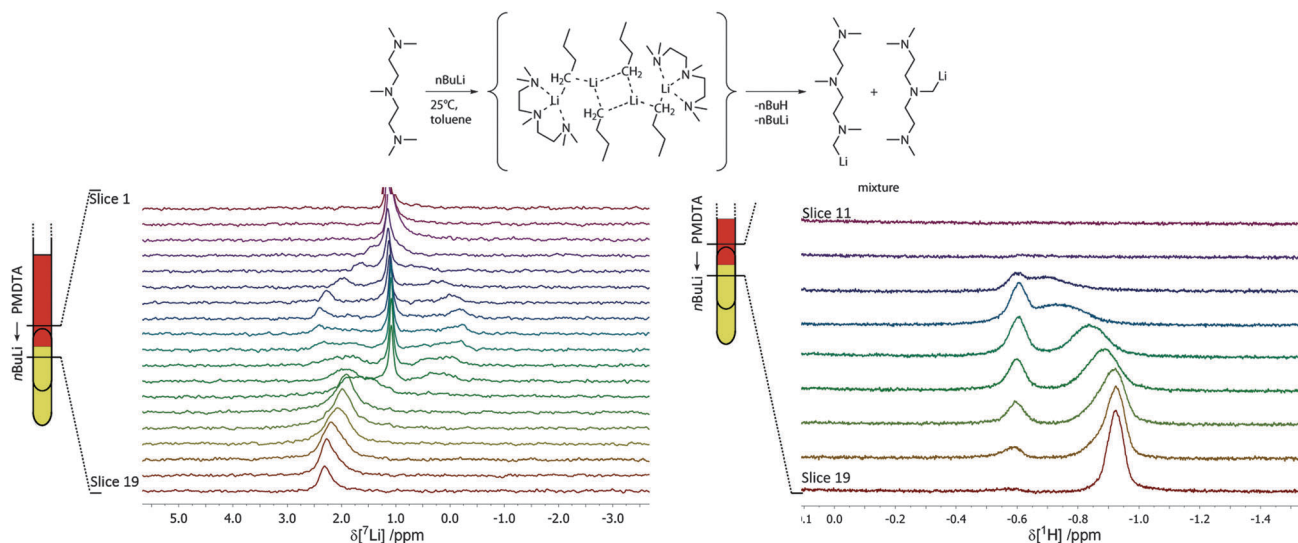
Fig. 2 (top) The reaction between nBuLi and PMDTA yields lithiated PMDTA *via* the intermediate [(nBuLi)$_2$PMDTA]$_2$. (bottom left) Slice-selective ^{7}Li NMR spectra (slices 1 to 19) of the reaction within a polystyrene gel, 3 days after the addition of PMDTA. (bottom right) Slice-selective ^{1}H NMR spectra (slices 11 to 19) of the same sample.
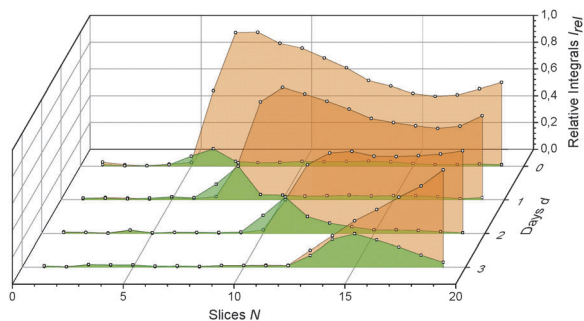
Fig. 3 Relative integrals for the ^{1}H NMR signals at −0.59 ppm (green) and that of the *n*BuLi α-CH$_2$ protons (orange) as function of the slice number, 3 h, 1, 2 and 3 days after addition of PMDTA to *n*BuLi inside the pre-swollen polystyrene gel. All signals high-field to −0.59 ppm were attributed to "*n*BuLi".

unspecific deaggregation and/or complexation of *n*BuLi by PMDTA. These processes are fast to intermediate on the NMR timescale such that only averaged chemical shifts are observed by ^{1}H and ^{7}Li NMR. From slice 12 onwards, the α-CH$_2$ region is free of signals indicating that *n*BuLi has completely reacted to butane.

The most remarkable feature in slices 18–13 is the appearance of an additional, unshifted ^{1}H resonance at −0.59 ppm with a maximum intensity in slice 15. This signal was assigned to the complex [(*n*BuLi)$_2$PMDTA]$_2$ (see Fig. 2 top), which seems to be remarkably stable and has been characterised by X-ray crystallography, ^{1}H NMR and quantum chemical calculations as an intermediate in the lithiation of PMDTA before.[21] Further ^{1}H signals reported for [(*n*BuLi)$_2$PMDTA]$_2$ were found in the crowded regions of the corresponding slices (see ESI†).

The connection between the reaction progress and the appearance of the peak at −0.59 ppm can nicely be illustrated by integrating this peak and the remaining *n*BuLi α-CH$_2$ signal over the course of three days. Fig. 3 illustrates the motion of the reaction front, where the *n*BuLi α-CH$_2$ signal (orange) vanishes and the signal of the corresponding CH$_2$-moiety within the [(*n*BuLi)$_2$PMDTA]$_2$ complex (green) appears. The latter builds up and decays exclusively at the reaction front, indicating its intermediate character. While 3 h after the addition the front is relatively sharp with little formation of [(*n*BuLi)$_2$PMDTA]$_2$, it becomes significantly blurred as it moves downwards with time. This is in accordance with what would be expected from diffusion in a gel.[24] The dip in the *n*BuLi concentration which is initially observed in the centre of the gel most likely arises from slow and incomplete diffusion of *n*BuLi into the polymer during sample preparation.[13] This again underlines that the *n*BuLi hexamer (M_w = 383 g mol^{-1}) may be regarded as rather static compared to the PMDTA molecules (M_w = 173 g mol^{-1}). No separate ^{7}Li NMR signal was found for [(*n*BuLi)$_2$PMDTA]$_2$, most likely due to fast dynamic exchange with the signal of *n*BuLi. Likewise, no evidence was found for further breaking down of [(*n*BuLi)$_2$PMDTA]$_2$ into smaller units representing active species/intermediates during the lithiation of PMDTA. Note that a monomeric *n*BuLi·PMDTA adduct has been proposed but its existence has not yet been experimentally confirmed.[25,26]

In the present study we could show that slice-selective NMR spectroscopy is a simple method to perform single-shot NMR titrations and *in situ* observation of reactions, using a routine

NMR instrument. The "fast titration" was successfully tested in the complexation of a lithium salt by 12-crown-4, where it was able to reproduce a conventional [7]Li chemical shift titration curve. Reaction monitoring by slice-selective NMR was tested in the reaction between PMDTA and *n*BuLi, where the previously characterised intermediate [(*n*BuLi)$_2$PMDTA]$_2$ could be identified. A stretched polystyrene gel was used as medium (i) to slow down the reaction and avoid convection, (ii) to immobilise one reactant (*n*BuLi) with respect to the other (PMDTA) and (iii) to principally enable also the observation of [7]Li RQCs as additional source of structural information. Polystyrene is chemically inert, tolerates highly reactive reagents,[20] and is swollen by a broad range of solvents.[27] A further advantage of the gel method is the possibility to adjust the slope of the reaction front and hence to "zoom" into the interesting region. It should therefore be possible to apply slice-selective NMR to a broad range of reactions to obtain information about mechanisms as well as stoichiometry of complexes and products.

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