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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 nBuLi with 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 intermediates4,5 has become possible through custom build NMR hardware and techniques such as stopped flow6,7 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 radiofrequency pulses with variable frequency offsets in the presence of a magnetic field gradient along the axis of the NMR tube.3,13 For highly sensitive nuclei such as 1H, 7Li, 19F or 31P, 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 LiClO4 (24 mg, 2.3 mmol) in acetonitrile-d3 (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 LiClO4 solution resulting in a smooth concentration gradient along the tube axis. Diffusion of LiClO4 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 Supplementary Information for details).13

Figure 1. Slice-selective 7Li NMR spectra of LiClO4 in acetonitrile-d3 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/LiClO4 concentration ratio is indicated on the right and increases from slice 1 to slice 19.

For each slice, absolute integrals of both the 7Li resonance of LiClO4 and the 1H resonance of 12-crown-4 were measured and

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converted into concentrations using homogenous reference samples. This way, the $^7$Li chemical shift observed in each slice can be specified to a specific ether/lithium ratio.

Figure 1 shows the series of 19 slice-selective $^7$Li NMR spectra recorded after 6 h (for spectra recorded after 3 and 9 h see Supplementary Information). Additional slice-selective $^1$H spectra (see also Supplementary Information) 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 $^7$Li resonance at -2.3 ppm, typical for Li$^+$ coordinated by four acetonitrile molecules. As the ether concentration increases, the $^7$Li 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. 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 can be overcome by further decreasing the slice-width. In general, a decrease in slice-width improves the resolution along the tube axis, but may lead to a sensitivity penalty which makes less intensive signals undetectable.

In the presence of an excess of 12-crown-4, the $^7$Li resonance is then shifted again upfield until it reaches -1.2 ppm in slice 19 (7-fold excess, [Li(12-crown-4)]$^+$). The resulting titration curve (see Supplementary Information) fully agrees with a previous report.

The application of slice-selective NMR spectroscopy to reaction monitoring is demonstrated with the reaction between $n$BuLi and PMDTA. 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. Slices 1 to 5 are located outside the polymer and hence only a singlet is observed for lithiated PMDTA in this region.

Figure 2 shows the slice-selective $^7$Li NMR spectra three days after the addition of PMDTA to the polymer imibed with $n$BuLi (for spectra recorded after 3 h, 1 and 2 days, see Supplementary Information). Slices 19 and 18 at the bottom of the active volume still show a signal for unreacted $n$BuLi at 2.3 ppm, in agreement with the absence of $^1$H signals of PMDTA in these slices (see also Supplementary Information). Satellite peaks in slices 6-12 arise from residual quadrupolar couplings (RQCs) due to partial orientation of molecules inside the stretched gel. 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.

Increasingly broad signals in slices 17-13 with chemical shifts between 2.3 and 1.6 ppm mark the reaction front between PMDTA and $n$BuLi, at which multiple dynamic processes take place. 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 $n$BuLi $\alpha$-CH$_2$ protons (-0.5 to -1 ppm) is quite informative (Figure 2 bottom right). The signal for the $n$BuLi hexamer at -0.92 ppm moves downfield and broadens from slice 19 to 13, presumably due to unspecified deggregation 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 $^3$Li NMR. From slice 12 onwards, the $\alpha$-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 [(nBuLi)$_2$PMDTA]$_2$ (see Figure 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. Further $^1$H signals reported for [(nBuLi)$_2$PMDTA]$_2$ were found in the crowded regions of the corresponding slices (see Supplementary Information).

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 nBuLi $\alpha$-CH$_2$ signal over the course of three days (Figure 3).

![Figure 3. Relative integrals for the $^1$H NMR signals at -0.59 ppm (green) and that of the nBuLi $\alpha$-CH$_2$ protons (orange) as function of the slice number, 3 h, 1, 2 and 3 days after addition of PMDTA to nBuLi inside the pre-swollen polystyrene gel. All signals high-field to -0.59 ppm were attributed to “nBuLi”.](image)

Figure 3 illustrates the motion of the reaction front, where the nBuLi $\alpha$-CH$_2$ signal (orange) vanishes and the signal of the corresponding CH$_2$–moiety within the [(nBuLi)$_2$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 [(nBuLi)$_2$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.

The dip in the nBuLi concentration which is initially observed in the centre of the gel most likely arises from slow and incomplete diffusion of nBuLi into the polymer during sample preparation. This again underlines that the nBuLi hexamer (MW = 383 g/mol) may be regarded as rather static on the NMR timescale such that only averaged chemical shifts are observed by $^1$H and $^3$Li NMR. From slice 12 onwards, the $\alpha$-CH$_2$ region is free of signals indicating that nBuLi has completely reacted to butane.

Further $^1$H signals reported for [(nBuLi)$_2$PMDTA]$_2$ were found in the crowded regions of the corresponding slices (see Supplementary Information).

Conclusions

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 $^3$Li chemical shift titration curve. Reaction monitoring by slice-selective NMR was tested in the reaction between PMDTA and nBuLi, where the previously characterised intermediate [(nBuLi)$_2$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 (nBuLi) with respect to the other (PMDTA) and (iii) to principally enable also the observation of $^3$Li RQCs as additional source of structural information. Polystyrene is chemically inert, tolerates highly reactive reagents, and is swollen by a broad range of solvents. 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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Notes and references