Methods and procedures for gel-phase HR MAS spectroscopy

Solution NMR spectra were acquired on a 300 MHz Bruker AC-300P FT spectrometer at 303 K. HR MAS NMR spectra were acquired on a Bruker DRX400 spectrometer at room temperature or below using a Bruker HR MAS microprobe. Rotors containing a suspension of the beads in $CDCl_3$ were spun at 4 kHz. One-dimensional HR MAS spectra were obtained with 64 scans. CPMG pulse sequence contained 32 or 2000 π-pulses with a repetition time of 30 ms. Chemical shifts (δ) are reported in parts per million relative to residual solvent.

It should be noted that some caveats are necessary in the use of this technique. A significant disadvantages of using HR MAS NMR spectroscopy to analyse solid-supported supramolecular systems, is the lack of accurate quantitation methods. This is due to two main factors; firstly, that the HR MAS NMR spectrum cannot be integrated as with standard ^{1}H NMR, due to the application of the CPMG pulse sequences used. While the CPMG pulse loops successfully filter out any broad resonances due to the bead core, this is indiscriminate and the intensity of signals arising from the tethered components can also be affected, depending on their relaxation properties. Signals broadened by relaxation or exchange processes are particularly affected, sometimes to such an extent that they are not observable in the filtered spectrum. Even relative integrations of individual protons within a single molecule, which are affected by the CPMG pulse sequences, can be anomalous. For example in the tethered pyridyl diimide tethered thread **5**, the four pyridine protons are filtered to varying degrees, and therefore do not have the expected 1:1:1:1 integration. Thus integration cannot be relied upon as an accurate indication of the relative loading of different components, and even strategies that might be used which incorporate an 'internal standard' marker proton resonance are not feasible. A more reliable internal comparison of relative integrations can be obtained from the unfiltered spectrum, but this is only feasible for those signals which are not obscured by the large and broad residual peaks arising from the structural components of the beads.

The second factor that renders any quantitation difficult, is that the loading of the tethered component on the bead is difficult to ascertain. The average percentage of functional sites on the beads is given by the manufacturer (around 0.46% mmol OH/g for ArgoGel beads), however, it cannot be assumed that the tethering reaction is 100% effective, either through incomplete reaction in the heterogeneous system, or restricted access (especially of larger reactants such as porphyrins) to available sites on the beads. Thus other indirect methods to determine the loading of components on the beads are required.

One such indirect method was by a UV Vis spectroscopic approach. By comparing the UV Vis spectrum of a solution of known concentration of rhodium porphyrin before and after the addition of a known quantity of beads, it is possible to estimate the amount of porphyrin coordinated to the terminal pyridyl groups on the tethered diimide thread, as any coordinated metalloporphyrin would be effectively removed from solution by absorption on the beads. Preliminary investigations indeed showed that the addition of beads to a solution of porphyrin of known concentration decreased the initial rhodium porphyrin UV absorption intensity. However, this decrease was found to be concentration dependent, and the relative decrease in porphyrin absorbance varied with the initial concentration of porphyrin solution. Control experiments showed that the addition of unfunctionalised beads to a solution of rhodium porphyrin of known concentration also resulted in a decrease in the expected porphyrin absorbance. This indicates some additional interaction between the porphyrin and the bead surface, which could be due to π-π stacking between the porphyrin and the polystyrene aromatics, or even by rhodium coordination to the exposed hydroxyl or ether groups of the beads (albeit weaker than with the

nitrogenous ligands of the attached thread). Thus, alternative strategies to determine the loading were needed.

The first method used two solutions of rhodium porphyrin of identical concentration. To one solution were added unfunctionalised beads, and to the other, the diimide thread functionalised beads **5**. The difference between the absorbance of the rhodium porphyrin between these two solutions thus gives an approximation for the amount of porphyrin coordinated to the pyridine group in the diimide tethered beads, compared to the concentration of porphyrin absorbed by the bead core. The loading of the porphyrin on the beads using this method was determined to be 0.22 mmol/g based on dry bead weight, and this value could be obtained reproducibly with varying porphyrin concentration

A converse method was also used. In this case the diimide thread-attached beads **5** and the unfunctionalised beads that had been pre-soaked in a concentrated porphyrin solution were filtered and both sets of beads were washed thoroughly and repeatedly (with CH_2Cl_2 and hexane, which causes alternate shrinking and swelling of the beads) with identical amounts of solvent until no colour remained on the unfunctionalised beads. The diimide beads **5** retained a red colouration due to the strong coordination of the rhodium porphyrin to the terminal pyridyl groups of the thread. These beads were then washed with an excess of pyridine/CH_2Cl_2 solution until all coordinated porphyrin was removed from the beads, and the filtrate made up to a standard volume. The absorptivity of this solution was then recorded to determine the amount of rhodium porphyrin displaced from the diimide thread. The loading of the porphyrin on the beads using this method was determined to be 0.21 mmol/g.

Elemental analysis for the nitrogen content in the diimide tethered beads **5** was 0.56 % (average of 2 combustion experiments) which converted to a bead loading of 0.13 mmol/g, with an associated error, as calculated as the standard deviation between runs, of 20%. This loading is lower than that calculated by UV Vis methods, and this could be due to problems in the purification of solid supported systems; for example dust and other solid particulates cannot be removed from the bead sample, and this could result in lower nitrogen content readings. Thorough drying of the beads is also somewhat problematic, as residual solvent and moisture is strongly adsorbed. Nevertheless it can be assumed that the loading of the naphthodiimide tethered beads **5** is approximately 0.19 mmol/g (taken as an average of the three methods) which is approximately 40% of the estimated functional group loading (0.46 mmol/g) reported by the manufacturer.

The lower measured loading of the diimide component on the beads, as compared to the available hydroxyl sites for tethering is intuitively reasonable, given the expected reduced yields for the heterogeneous system, and the possibility of steric interference from adjacent sites and inaccessibility of others. Nevertheless it is clear that these loadings are adequate for HR MAS NMR analysis yielding good quality spectra under these conditions.

A further hindrance to accurate quantitation in this methodology is a function of the technique itself. In the measurement process, small quantities of dried beads are loaded into the rotor. Although the initial precise weight of added beads could be obtained, in the next stage where these beads are swollen by adding excess solvent or solution inside the rotor and the cap is inserted, inevitably some unknown quantity of excess beads are squeezed from the sample cavity. Likewise, there is no simple means by which a measured volume of known concentration of surrounding solution can be ascertained with certainty, as in the swelling and capping process, an unknown portion of this solution is invariably ejected and lost. Irreproducible packing of the swollen beads inside the sample cavity also means that the volume occupied by the surrounding

solution is variable from one sample to the next. Nevertheless, an element of reproducibility can be obtained by a consistent approach using similar quantities and concentrations of swelling solution, and an estimated similar bead sample size.

For those experiments where sequential addition of reagents are needed, it is impractical to add the second reagent to already swollen beads within the cavity of the rotor (since the beads and solvent are tightly confined within the tiny internal space of the rotor, and this is accessible only through a small orifice). Furthermore, the swelling process itself is a necessary function to maximise reagent access to the bead functionalised sites, and it is not feasible to remove, shrink, and re-swell beads from the cavity. Thus, in those cases where sequential or multiple reagent addition is required, separate samples must be made, one with the added first reagent, and the next with both reagents added concurrently in the surrounding solution (this can of course only be successful in systems such as those used in this study where all solid-phase and solution components are in equilibrium). A certain degree of subjectivity must then be exercised in the comparison of the two different samples, as the signal strength of both bead-attached and solution-phase components cannot be compared directly.