Quantifying an unknown concentration by calibrated titration
and integration of NMR peak areas
(YB, June 2003)

(1) The NMR tube contains an unknown amount of substance ***U*** to be quantified. The volume of material in the NMR tube will need to be precisely known (***V***). The spectral peaks of ***U*** must already be assigned and must be well resolved from any other peaks in the NMR spectrum. The NMR signal must be acquired under quantitative conditions.

$V_o$ → known initital volume
$U_o$ → unknown amount to be quantified

(2) A separate solution of the material ***U*** (or a suitable non-interacting reference) of known concentration (***C*** = ***U/***V) should be prepared.

(3) Acquisition hints for quantitative integrations:

Measure the longest $T_1$ and use $5*T_1$ for the recycle delay
Use a large sw to get a flat baseline
Center your data in the middle of the spectrum (o1)
Acquire a large number of dots (TD)
Make sure the signal-to-noise ratio is high (NS)
Use an optimal AQ time (fid going to zero at ~1/3 of the screen)
For decoupled measurements of X nuclei – you MUST avoid any NOE effects
Repeat for a few different independent measurements

(4) THE EXPERIMENT

***i.*** Acquire an FID, FT, and integrate the original sample containing $U_o/V_o$. If you expect that after titration the signal will be truncated, then reduce the receiver gain at this stage, BEFORE acquiring the reference FID.

***ii.*** add into the NMR tube an aliquot of known volume (***V***) of known concentration (***C)*** so you now have $(U+U_o)/(V_o+V)$. Acquire an FID, FT, and integrate – using all the same parameters of step 4***i*** (***i.e.*** do NOT do an rga . . .)

***iii.*** repeat step 4***ii*** for several aliquots of material. A minimum of three aliquots should be used to make the calibration curve.

(5) Processing hints for quantitative integrations:

Do not use a window function, just do a straight fourier transform
Be sure that the baseline is flat on both sides of the peaks to be integrated
Include the $^{13}C$ satellites in the integration
Adjust the slope and bias of the integration
Use the same limits of integration for all spectra (wmisc→ intrng, rmisc→intrng)
Use the same scale for all the integrations ("lastscal")
Use deconvolution software, if lines are not resolved

(6) To create a calibration curve, start with a table summarizing your NMR results:

| expno | $\boldsymbol{U}$ (μg) added | $\boldsymbol{V}$ (μl) added | $\boldsymbol{V_o+V}$ (μl) | conc of added ($\boldsymbol{C'}$) | Normalized peak area | Peak area of added |
|---|---|---|---|---|---|---|
| 1 | none | none | 500.0 | none | 1.00 | none |
| 2 | 100 | 1.5 | 501.5 | 0.2 (μg/μl) | 1.50 | 0.50 |
| 3 | 200 | 3.0 | 503.0 | 0.4 (μg/μl) | 3.88 | 2.88 |
| 4 | 300 | 4.5 | 504.5 | 0.6 (μg/μl) | 6.02 | 5.02 |

Then plot the peak area due to the added material versus the concentration added – this is your calibration curve.

Using the peak area of the material at its initial concentration equal to 1.00, the corresponding concentration can be determined directly from the graph.