

4.2: Background

There are a number of issues that should be considered when measuring NMR spectra for purposes of quantitative analysis. Many of these issues pertain to the way that the NMR data is acquired and processed. It is usually necessary to perform quantitative NMR measurements with care to obtain accurate and precise quantitative results. The advantages of NMR over other spectroscopic methods are that no response factor is needed and all the resonances generated by a particular nucleus (for example ^1H , ^{31}P or ^{19}F) have an integrated intensity directly proportional to the molar concentration of the analyte and to the number of nuclei that give rise to that resonance.

$$\frac{\text{Integral Area}}{\text{Number of Nuclei}} \propto \text{Concentration} \quad (4.2.1)$$

The ^1H NMR resonance of a methyl group would have 3 times the intensity of a peak resulting from a single proton. For example, sodium 3-(trimethylsilyl)tetrapropionate TSP, has a methyl resonance equivalent to 9 protons (from the three methyl groups) and therefore would give rise to a resonance that is 9 times greater than the intensity resulting from a single proton.

For this experiment, KHP (potassium hydrogen phthalate) will be used as an internal quantitation standard. KHP has the advantage of being a primary standard, meaning that after drying, its solution concentration can be calculated directly from its mass. You may also wish you use an internal chemical shift reference, like TSP- d_4 in the preparation of your solutions. TSP is not a primary standard, and is known to adsorb to glass surfaces which can change its solution concentration over time, therefore it is not a useful quantitation standard. As a chemical shift reference, TSP- d_4 has the advantage of producing a single sharp resonance with a defined chemical shift of 0.00 ppm.

Malic acid and citric acids are the major organic acids in fruits. Q-NMR is a valuable technique for determining the quantities of major and minor compounds in fruit juices. By comparing the resonance integral of an analyte to that of a standard compound of known concentration, we can determine the analyte concentration according to the equation below:

$$C_{\text{analyte}} = \frac{I_{\text{analyte}} \times C_{\text{std}}}{I_{\text{std}}} \quad (4.2.2)$$

where C_{analyte} is the analyte concentration, C_{std} is the quantitation standard concentration, and I_{analyte} and I_{std} are the areas of the resonances of the analyte and the standard, respectively, normalized to the number of protons giving rise to each resonance.

The accuracy and precision of the integral measurements are affected by the following experimental factors.

- spectral S/N
- line shape
- quality of shimming
- baseline
- apodization window functions
- phasing, baseline-, and drift -corrections

Resonance overlap is a potential problem in accurate quantitation by NMR. This problem can sometimes be solved by careful selection of pH, using a different solvent, or adding a reagent to change the analyte chemical shift (i.e. lanthanide shift reagents). In some cases, the ^1H NMR spectrum may be too crowded for accurate quantitation, but another nucleus, for example ^{19}F or ^{13}C , that has a larger chemical shift window might produce well-separated resonances of the mixture components.

Field-frequency lock

The fields of superconducting magnets tend to drift over a period of minutes to hours causing loss of resolution. Most modern spectrometers are equipped with a lock channel that regulates the spectrometer field by monitoring the chemical shift of a deuterium resonance of the solvent. As the magnetic field drifts, the change in the deuterium resonance frequency generates an error signal that indicates both the magnitude and the direction of the field change, allowing compensation by a feedback circuit. Non-viscous deuterated solvents provide the best field-frequency lock because of their sharp and intense resonances. In ^1H NMR experiments an additional advantage of preparing samples in deuterated solvents is that the intensity of the solvent proton resonance is reduced. The resonance of protic solvents (e.g., H_2O , or CH_3CN) can obscure analyte ^1H NMR resonances and reduce the dynamic range of the measurement. While it is common to suppress the ^1H NMR resonances of protic solvents, analyte resonances with similar chemical shifts will also be suppressed. Sometimes it is not possible to make the sample solution in a

deuterated solvent, for example when the sample is already a liquid (i.e. blood plasma, urine or fruit juice). In such a case, a sufficient quantity of a deuterated solvent, like 10% D₂O, is added to the sample to provide the lock signal.

Solvent Suppression

Apart from accurate tuning of the probe and pulse width calibration, effective suppression of the solvent resonance is often crucial for the analyte resonance to be observed in aqueous samples. There are a number of solvent suppression methods available for use in NMR experiments, the simplest of which is presaturation. Presaturation uses a selective pulse to equalize the populations of the solvent spins. It is important to evaluate the effect of the solvent presaturation parameters on the resolution and sensitivity of the experiment to ensure good results. The presaturation power should be selected such that the solvent resonance is significantly attenuated without reducing the intensity of neighboring analyte resonances.

Repetition Time

The time between successive acquisitions is crucial in Q-NMR. To determine the longitudinal relaxation delay of a given analyte proton, the inversion recovery pulse sequence is used to measure T₁ relaxation times. The pulse sequence uses a calibration program which fits data to the exponential decay equation

$$I(\tau) = I_0 \left(1 - 2 \times e^{-\frac{\tau}{T_1}} \right) \quad (4.2.3)$$

where I(τ) is the intensity of the selected proton resonance for a given τ value, I₀ the intensity at equilibrium (infinite τ), τ is the value of the inversion delay, and T₁ is the first order time constant for longitudinal relaxation.

Malic and citric acid content of fruits

The table below summarizes the results obtained from quantitative NMR analysis of malic and citric acid content of various fruits.¹

	Malic Acid	Citric Acid
Apple*	3.42-10.12 g/L	0.09-0.36 g/L
Apricot	4.59	4.13
Pear	2.55	1.05
Kiwi	2.66	11.00
Orange	2.13	11.71
Strawberry	1.74	7.13
Pineapple	1.33	5.99

*Data obtained from three apples ranged between the values given.

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