Quantification / qNMR

PERCH NMR Software Course

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Spectral Parameter Prediction

Overview

- Methods
- Linear & Non-Linear Approaches
- Prior Knowledge
- Applications
- Examples

Spectroscopic Methods

- UV, VIS, IR, Fluorescence
- HPLC, GC, LC
- MS
- NMR

Time Domain

Frequency Domain

Time vs. Frequency Domain

- Time Domain
  - Raw data
  - No processing artifacts
  - Difficult to implement prior knowledge
  - Difficult to comprehend
- Frequency Domain
  - Processed data (windowing, phasing)
  - Processing artifacts
  - Easy to implement prior knowledge
  - Easy to visualize

Linear & Non-Linear Approaches

- Linear
  - Linear prediction (time domain)
  - Integration (freq. domain)
  - Histograms
- Non-Linear
  - Non-linear fitting in the time-domain (VARPRO)
  - Total-Line-Shape Fitting (freq. domain)
  - Other iterative approaches
Discrete - Continuous Lines

Integration

Overlapping Signals

Histograms (Buckets)

Deconvolution

Spectral Parameters
- Frequency
- Intensity
- Line Width
- Line Shape
- Baseline
Trial Spectrum

Observed
Calculated
Difference

Total-Line-Shape Fitting

Overlap between both spectra
\[ \delta I_i \approx 0 \]
\[ \delta P_i \]
Goal: Minimize \( SQ = \sum_i (I_{i, \text{obs}} - I_{i, \text{cal}})^2 \)

Newton-Raphson Iteration

In the Newton-Raphson iteration we must find the values of the nonlinear equation

\[
\frac{df}{dx} = \frac{\partial f}{\partial x} \cdot \frac{\partial x}{\partial x} + \frac{\partial f}{\partial y} \cdot \frac{\partial y}{\partial x} + \cdots
\]

and

\[
\frac{df}{dy} = \frac{\partial f}{\partial x} \cdot \frac{\partial x}{\partial y} + \frac{\partial f}{\partial y} \cdot \frac{\partial y}{\partial y} + \cdots
\]

The iterated scheme is:

\[
\begin{align*}
I & = I_0 + \delta I \\
\hat{I} & = \hat{I}_0 + \delta \hat{I}
\end{align*}
\]

Line-Shape

Lorentzian %
Gaussian %

300
250
200
150
100
50
0
-50
-100
-150
-200

A Simple Example
Prior Knowledge

- Frequency
- Intensity
- Line-width
- Line-shape
- Areas
- NMR multiplets
- and any linear combination of the above

Quantification by Deconvolution

13C-1H Lactate Isotopomers:

33 Lines

Application of qNMR

- Drug impurity analysis
- Bio-Fluids (Medical Diagnostics)
- Metabonomics
- Protein-ligand interactions
- Lipid peroxidation
- T1 Measurement

Disease Status Prediction from 1H NMR Spectra
Deconvolution of Multiple Spectra

An inversion recovery experiment on a methyl region of cyclohexane-A shows the use of constraints in the deconvolution of multiple spectra. The frequencies of each single line, the line-widths of each multiplet, and also the coupling constant of the triplet are constrained throughout the analysis.

Quantification by Deconvolution

\(^{13}C\)-H Lactate Isotopomers:

- \(^{13}CH_3CHOH\)
- \(^{13}CH_2CD_2CHOH\)
- \(^{13}CH_2CHOH\)
- Prior Knowledge
- Neutral Loss
- CI
- Neutral Loss
- Total Molecularity
- For each multiplet line: Same coupling splitting, same line-widths & line-shape

Linear vs. Iterative Approach

\[ \frac{\partial L}{\partial \mu_i} = 0 \quad \frac{\partial L}{\partial P_i} \]

Total-Line-Shape Fitting - Minimize SQ = \[ \sum \left( I_{\text{obs}} - I_{\text{calc}} \right)^2 \]

Solvent Signal Removal

Multiplet constraints used to deconvolute an acetone solvent signal overlapping with parts of the spectrum:

Subtraction of the solvent signal:

Spectrum without the solvent signal:

NMR Metabo*ics a la PERCH

qNMR Strategies

- Linear Approach
  - Spectral Libraries, Buckets
- Constraint Total-Line-Shape Fitting (CTLS)
  - Frequencies, Intensities, Signal Areas
  - Couplings (Multiplets)
  - Line-Shape Parameters
  - Any linear combination of the above
- Quantum Mechanical TLS Fitting (QM-TLS)
  - Adaptive Spectral Libraries: Field, pH,...
  - Integral Transforms
Metabolite Database

Field Strength

Line-Width

pH-Dependency

Adaptive Spectral Database

- Completely assigned & verified
- Free of artefacts, impurities etc.
- Flexible adaptable to any parameterized condition (field-strength, solvent, pH, etc.)
- Minimum storage space (just parameters)
- Fast calculation

Applications?

CONCLUSIONS

- NMR is a versatile quantification tool with many potential applications in chemistry and biosciences (both in-vitro & in-vivo).
- With modern instrumentation & software, the method rivals the traditional methods like HPLC also in economy – extensive calibration is not necessary.