Getting started with NMR

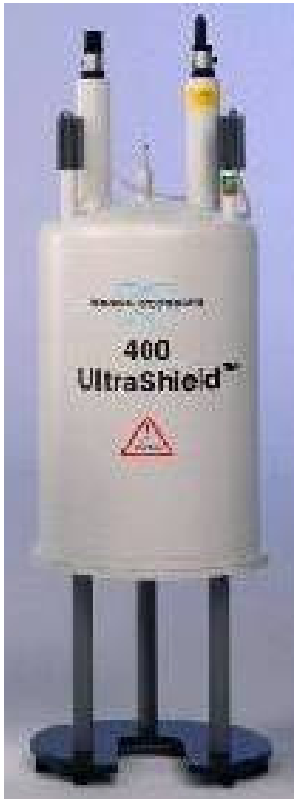
Practical Introduction

- ➔ Hardware
- ➔ NMR sample preparation
- ➔ starting on the NMR spectrometer
- ➔ troubleshooting
- ➔ pulse calibration

rak jan 2001

HARDWARE

BRUKER AVANCE DRX

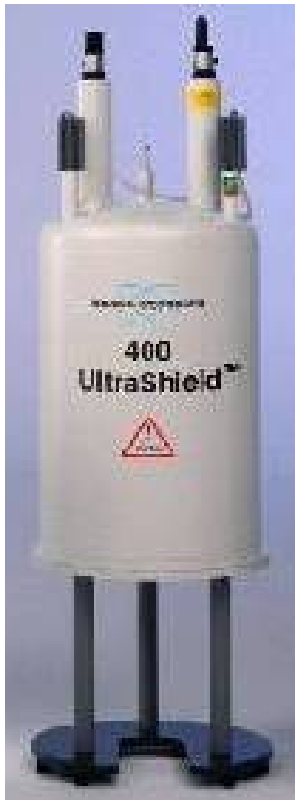


Magnet

Console

Host Computer

HARDWARE - MAGNET

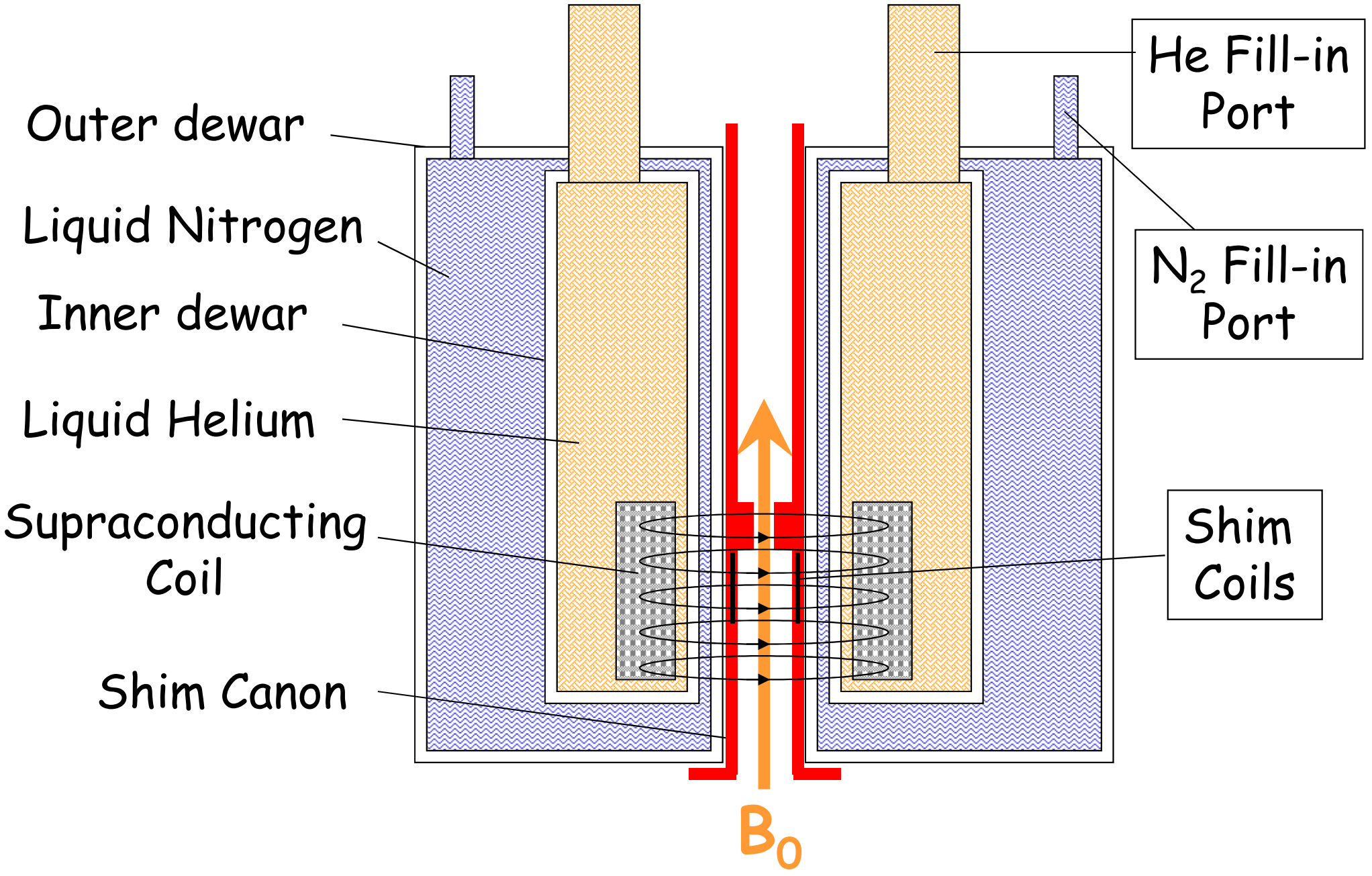


STRONG MAGNETIC FIELDS!!

For Work on NMR Spectrometers:

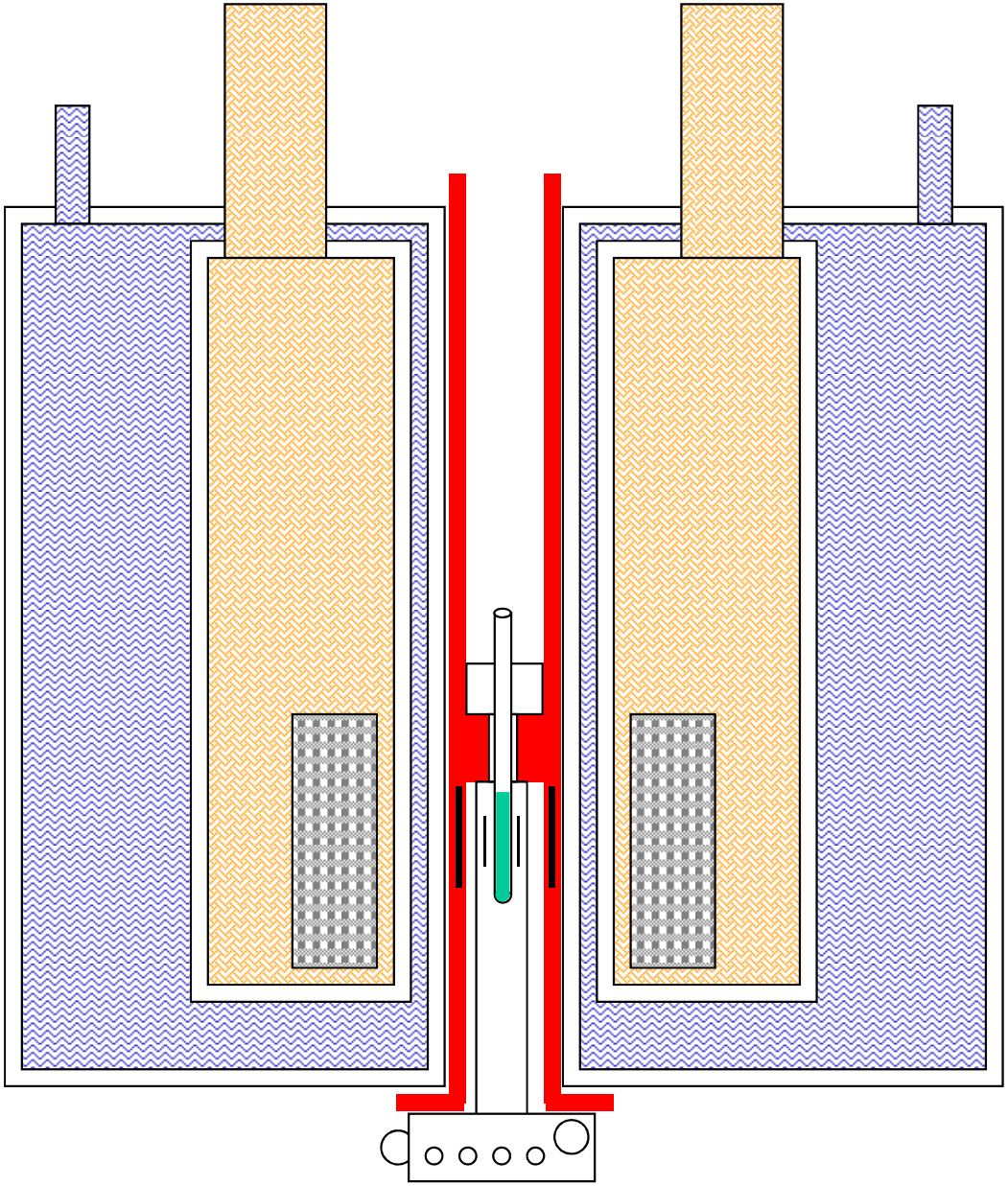
- NO PACE MAKERS!
- NO FERROMAGNETIC MATERIAL (keys, screw drivers, …)
- NO MAGNETIC CARDS (bancomat,…)
- NO ELECTROMAGNETIC FIELDS
- NO WATCHES
- NO DATA STORAGE DEVICES (magneto-optic discs, floppy discs, tapes, …)

HARDWARE - MAGNET



rak jan 2001

MAGNET and PROBEHEAD



rak jan 2001

NMR SAMPLE PREPARATION

Volume for 5mm tubes:

standard tube: at least 400µl, better 600µl

shigemi tube: at least 20mm volume height (~ 250 µl)

Buffer and Salts:

buffer without protons (KPi)

lowest concentration to obtain stable sample (20 mM)

avoid bubbles

1) Use the Correct Quantity of Material.
If about 0.2 to 0.3 millimoles can be dissolved in 0.7ml, the spectrum will take no more than about half an hour to record. If the quantity of material is halved, the data accumulation time will be quadrupled.

2) Remove All Solid Particles.
Solid particles distort the magnetic field homogeneity because the magnetic susceptibility of a particle is different from that of the solution. A sample containing suspended particles thus has a field homogeneity distortion around every single particle. This causes broad lines and indistinct spectra that cannot be corrected. So that there are no solid particles in your samples, you must filter ALL samples into the nmr tube.

3) Make Samples to the Correct Depth.
Sample depth must be between 4.5cm and 5.5cm. Shorter samples are very difficult to shim, and cause considerable delay in recording the spectrum. Samples that are too long are also difficult to shim and are a waste of costly solvent. You should check your sample depth using a ruler. After preparation, you should ensure that the cap is pushed fully onto the tube to minimise solvent loss through evaporation.

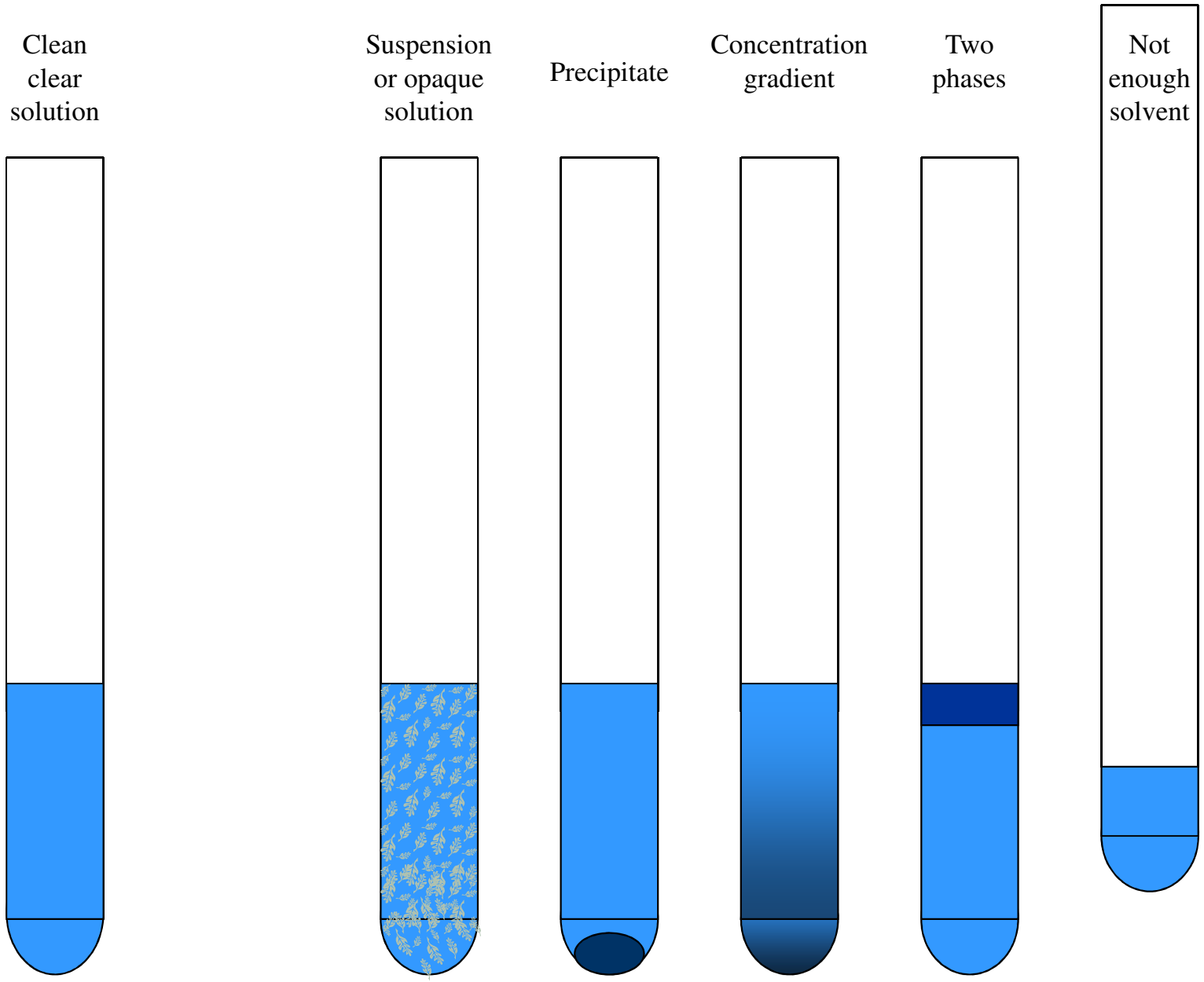
4) Use Deuterated Solvents.
Samples must be prepared using solvents that contain deuterium in place of hydrogen. The NMR signal from the deuterium nuclei is called the NMR lock and is used by the spectrometer for stabilization.

5) Use Clean Tubes and Caps.
NMR tubes should be rinsed with acetone or some other suitable solvent, then dried with a blast of dry air or nitrogen. Do NOT dry tubes in a hot oven because it does not remove solvent vapour effectively, and solvent peaks will appear in your spectrum. Tubes must be capped, and caps should be treated the same way as tubes. You must not use NMR tubes with a chipped or broken top because they are dangerous, and very likely to splinter lengthwise.

6) Label Your Samples.
This is best done with a permanent marker directly on the top of the tube, or on the cap. If you use a sticker or a piece of tape, your label must stick smoothly on the tube.

7) Use an internal reference.

8) Degassing Samples.
Some samples need to be degassed or have oxygen removed.



GOOD!

Bad Samples!

NMR SAMPLE PREPARATION

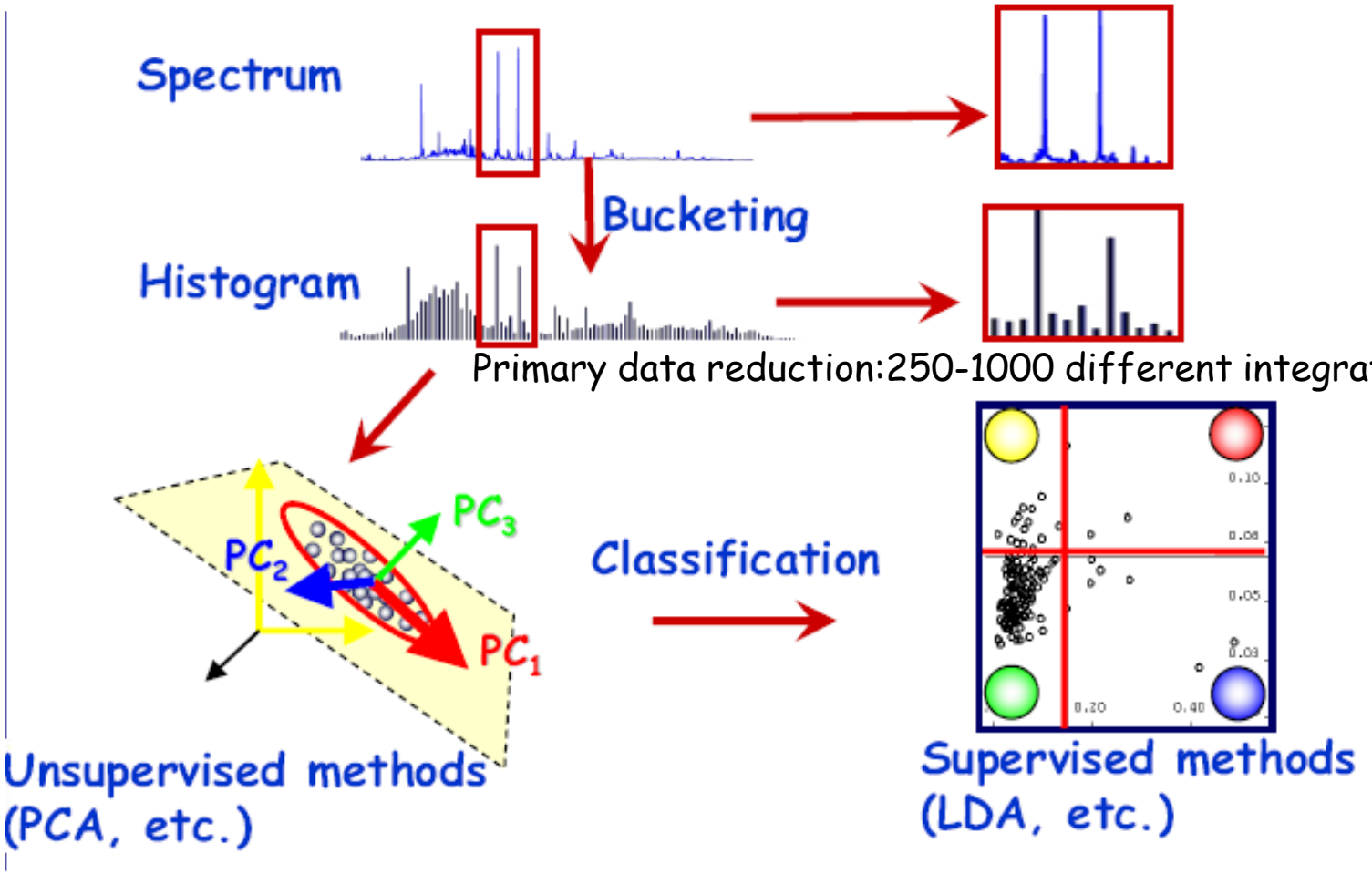
pH

to obtain exchangeable protons: lowest possible (5-6)

Oxygen free preparation

best: glove box

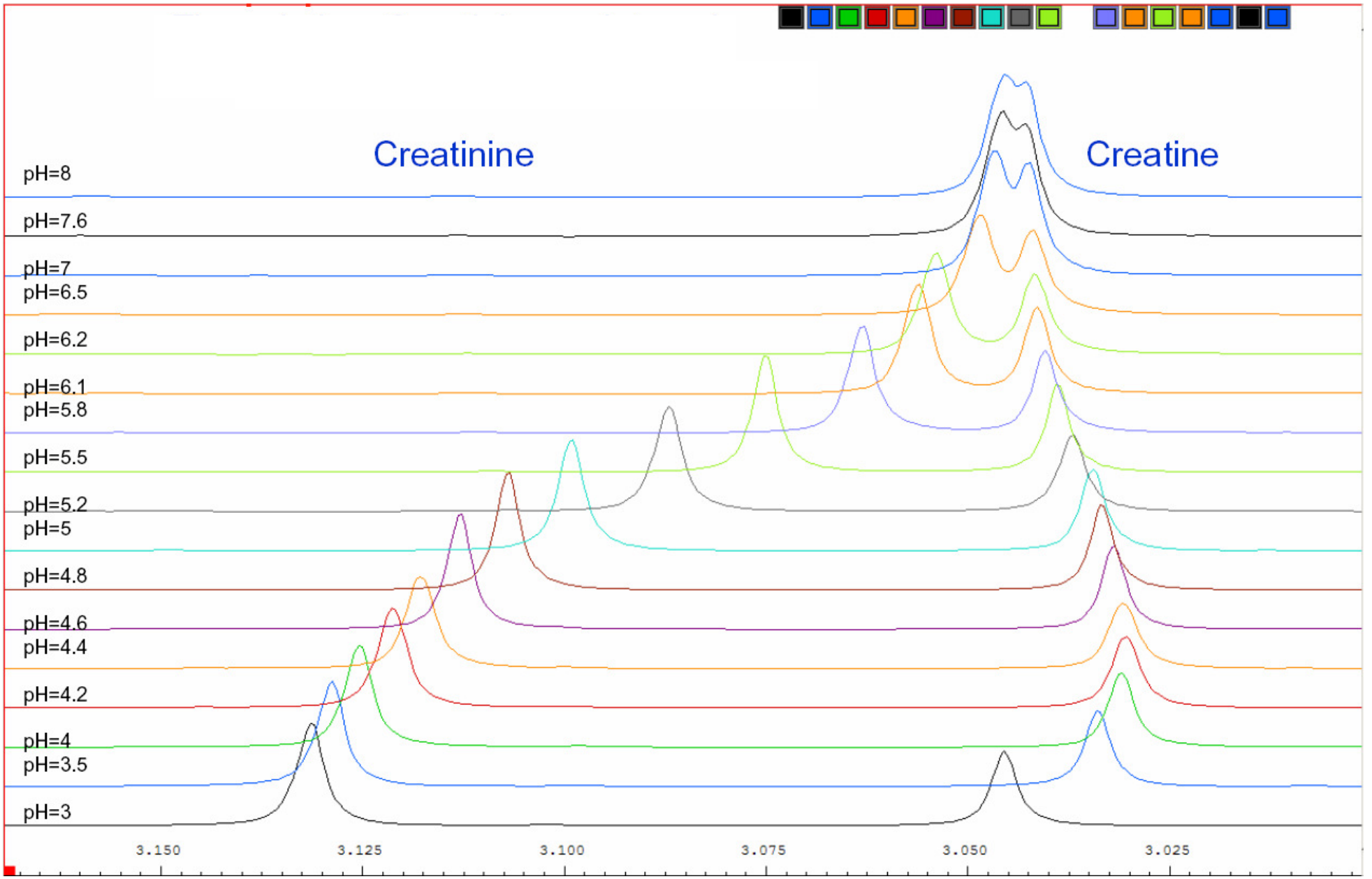
NMR-based metabolomics: the concept



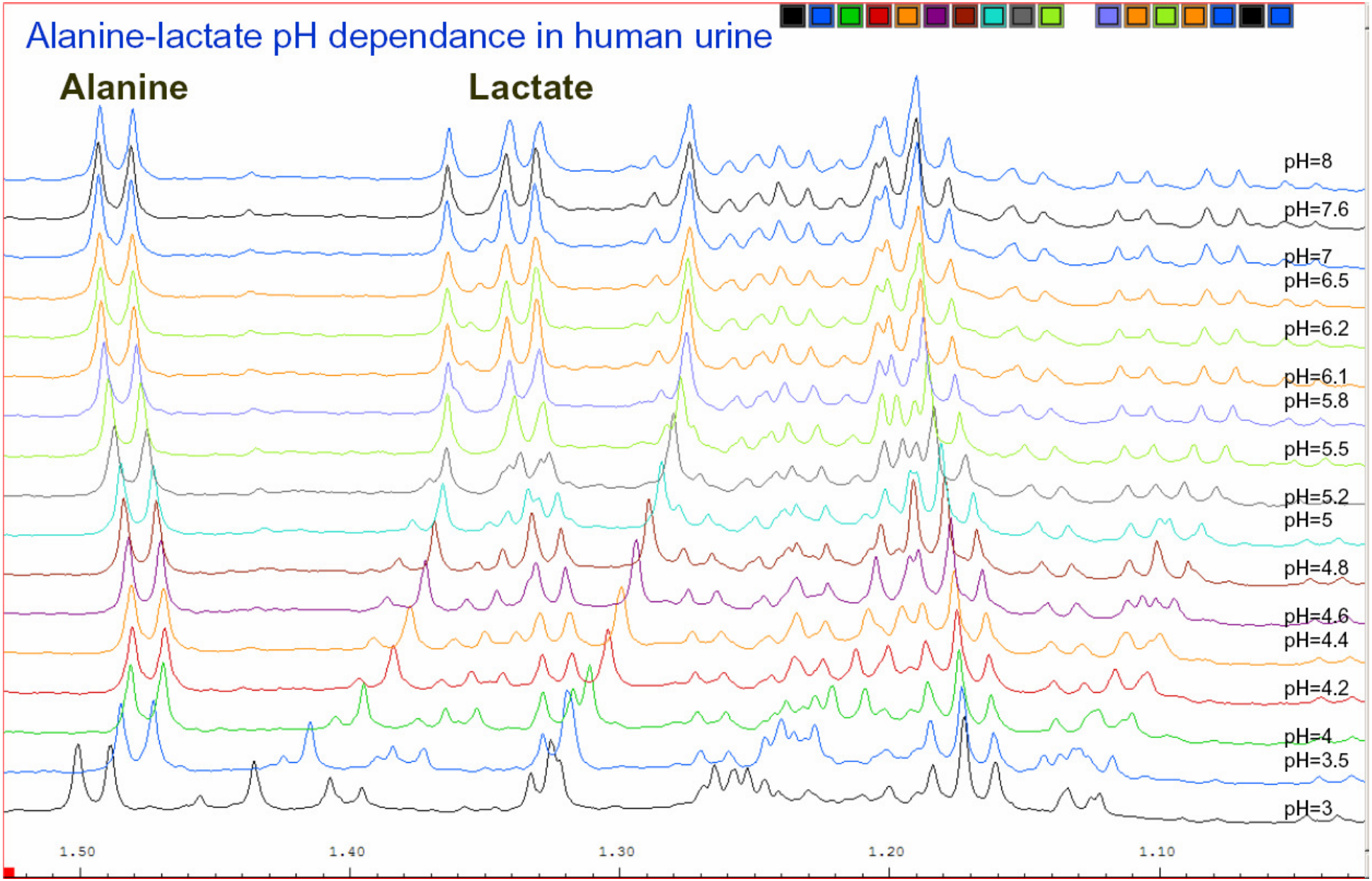
No *a priori* knowledge of the class of samples

Model for the prediction of independent data
Use class information to maximise separation among classes

pH changes


Creatinine
Creatine
pH=8
pH=7.6
pH=7
pH=6.5
pH=6.2
pH=6.1
pH=5.8
pH=5.5
pH=5.2
pH=5
pH=4.8
pH=4.6
pH=4.4
pH=4.2
pH=4
pH=3.5
pH=3
3.150
3.125
3.100
3.075
3.050
3.025

pH changes (…)

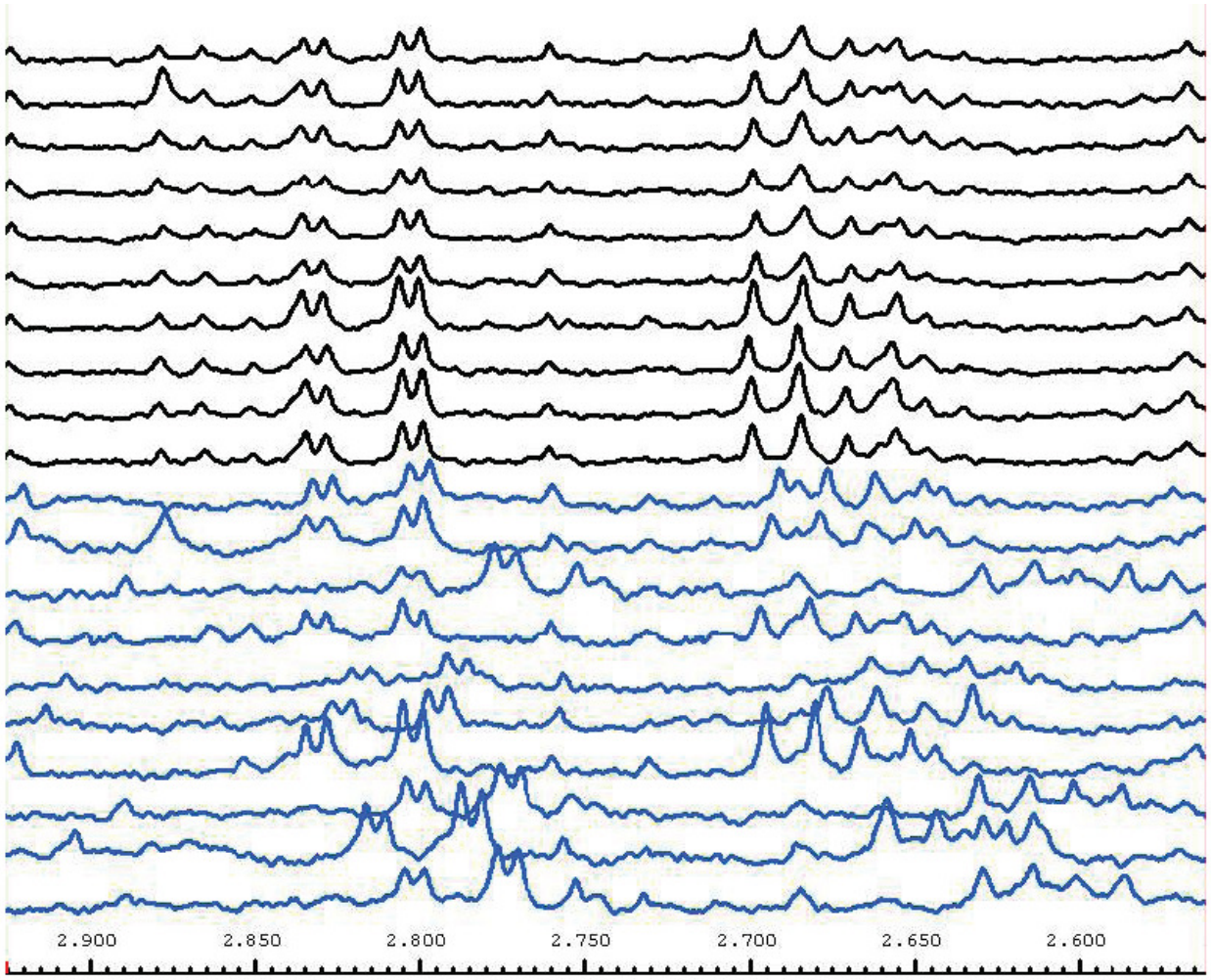

Alanine-lactate pH dependance in human urine
Alanine
Lactate
pH=8
pH=7.6
pH=7
pH=6.5
pH=6.2
pH=6.1
pH=5.8
pH=5.5
pH=5.2
pH=5
pH=4.8
pH=4.6
pH=4.4
pH=4.2
pH=4
pH=3.5
pH=3
1.50
1.40
1.30
1.20
1.10

pH adjustment

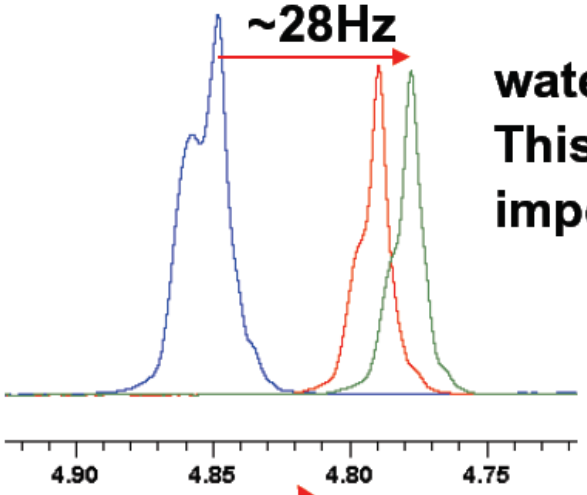
Buffer + pH adjustment
(pH = 7)

Buffer:
1.5M phosphat buffer
(KH2P04) in D20.
~0.01% NaN3 and
0.1% TSP is added.

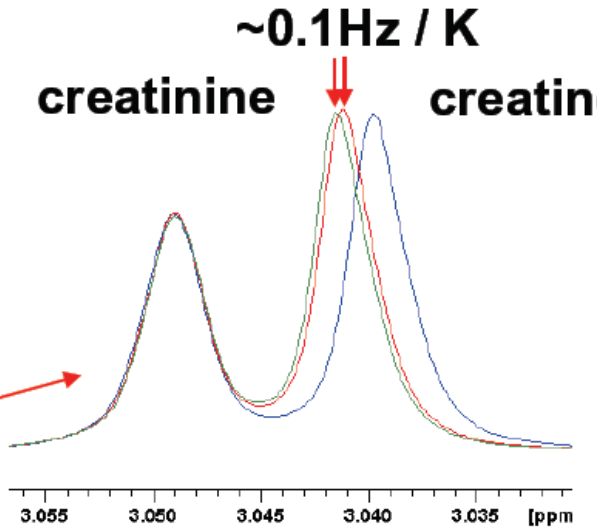
extreme cases most affected region



Temperature effects

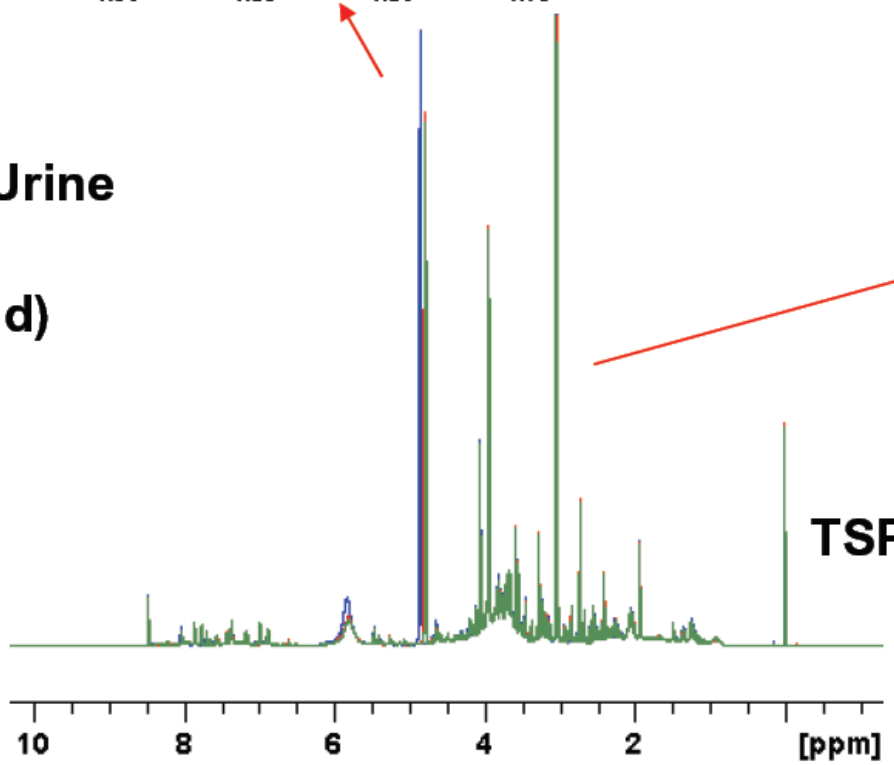


water: ~4.5 Hz / K!
This is the most important effect!!!

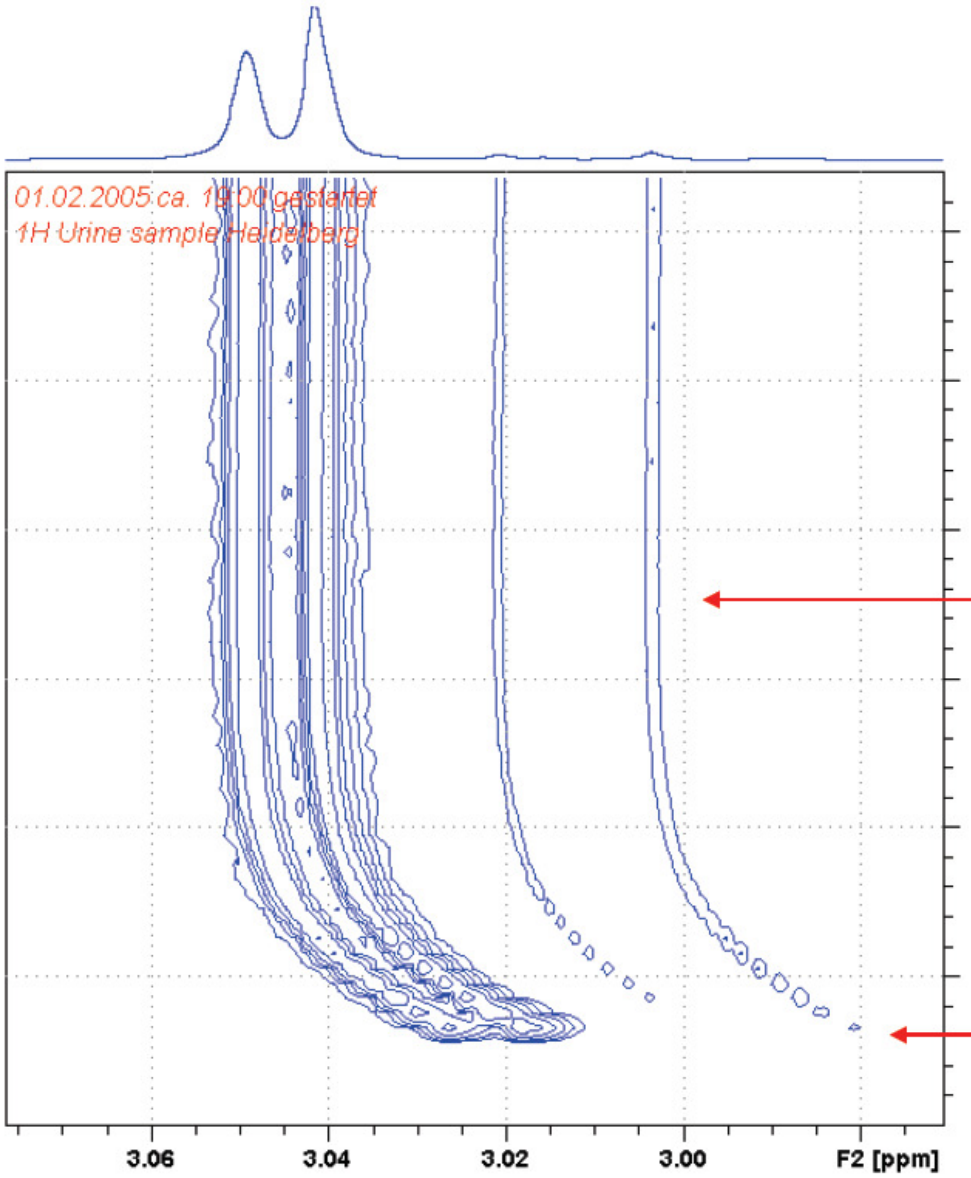


1H NMR on Urine
@ 400 MHz
(noesygppr1d)

295K
300K
301K



Temperature equilibration



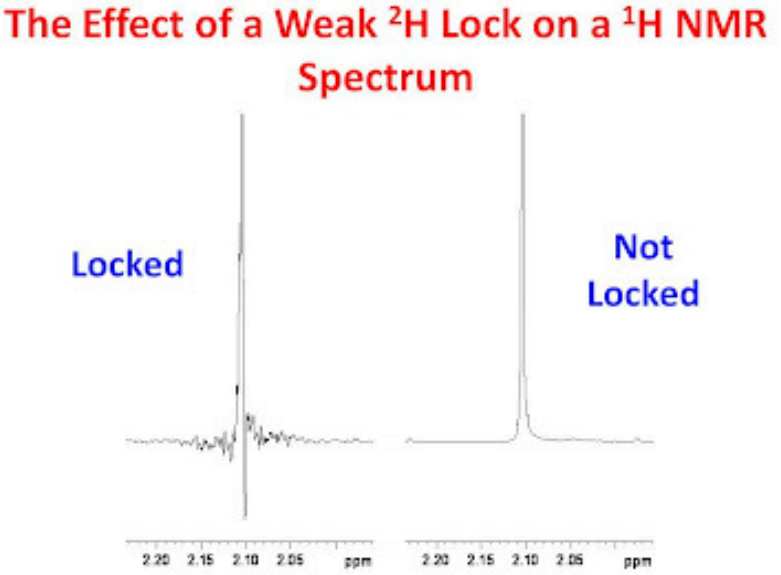
temperature equilibrated

5 min

sample dropped Into magnet

Before acquisition:
Frequency-field lock

Stability of the magnetic field is achieved by the *deuterium lock* system. The deuterium lock measures the frequency of the deuterium line of the solvent. Hence, deuterated solvents have to be used for FT-NMR. The system has a feedback loop, which generates corrections to the magnetic field strength B_0, such that the resonance frequency of the solvent deuterium line remains constant. This is achieved by delivering a suitable current to the z^0 shim coil. Consequently, all other resonance frequencies are also kept constant. Usually the lock system has to be activated when the sample has been placed in the magnet. When the lock-system is not activated the naturally occurring drift of the magnetic field leads to varying resonance frequencies over time and hence to line-broadening.



Before acquisition:
Probehead tuning/matching

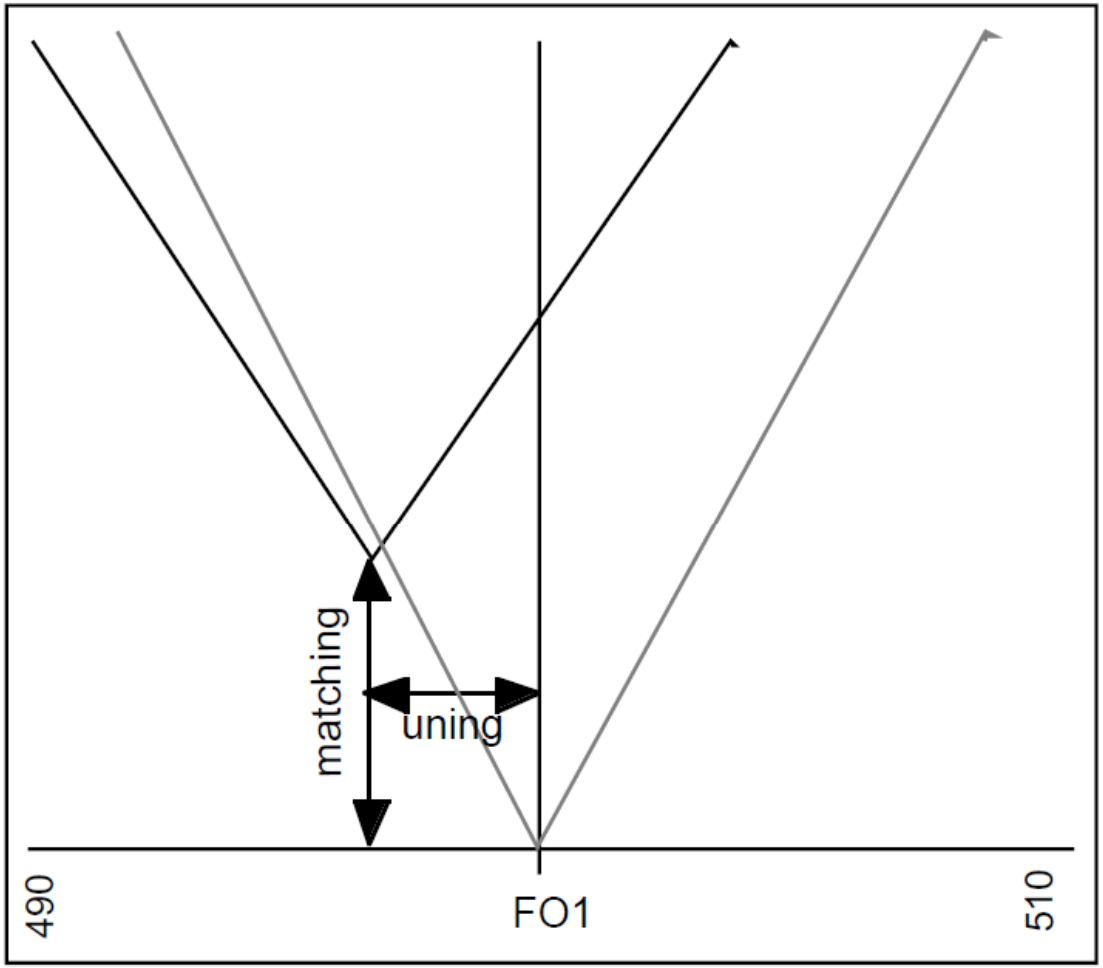


FIGURE 5. Wobbling curve of a detuned probe (thick line). By tuning, the minimum is moved along the horizontal axis and by matching the minimum becomes deeper. The optimum setting is shown as a dotted line.

Before acquisition: shimming

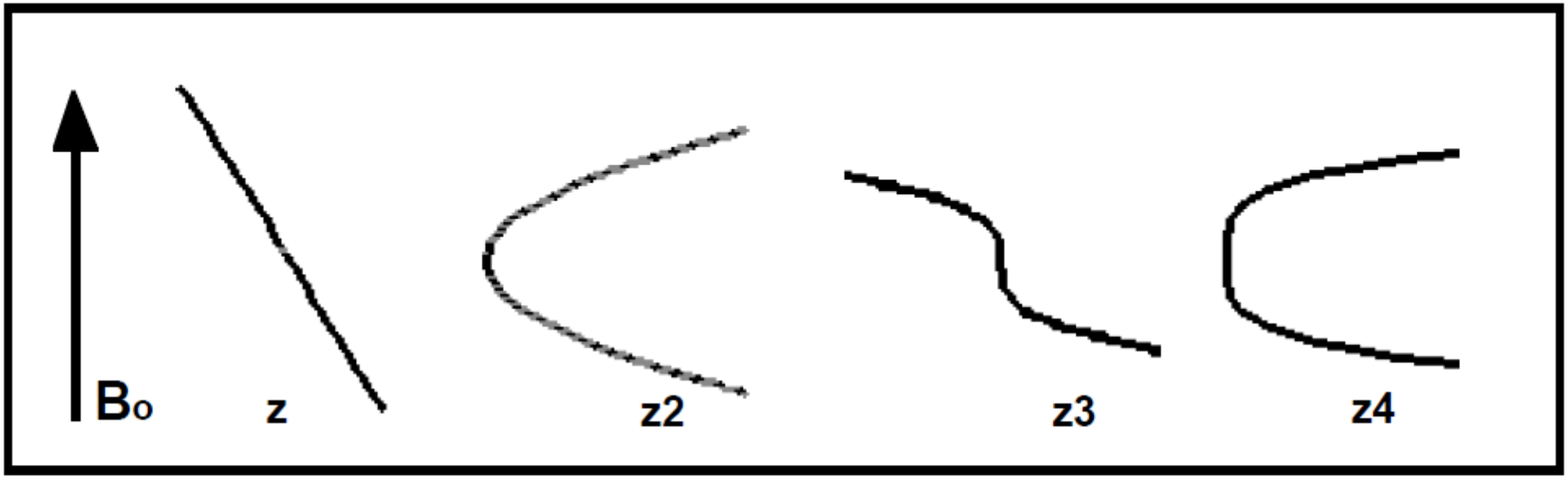


FIGURE 4. Field dependence of on-axis shims

The z-shim delivers an additional field that linearly varies along the sample tube. The z^2 shim has its largest corrections to the field at the top and the bottom of the sample.

Before acquisition: Shimming
(adjusting homogeneity of magnetic field)

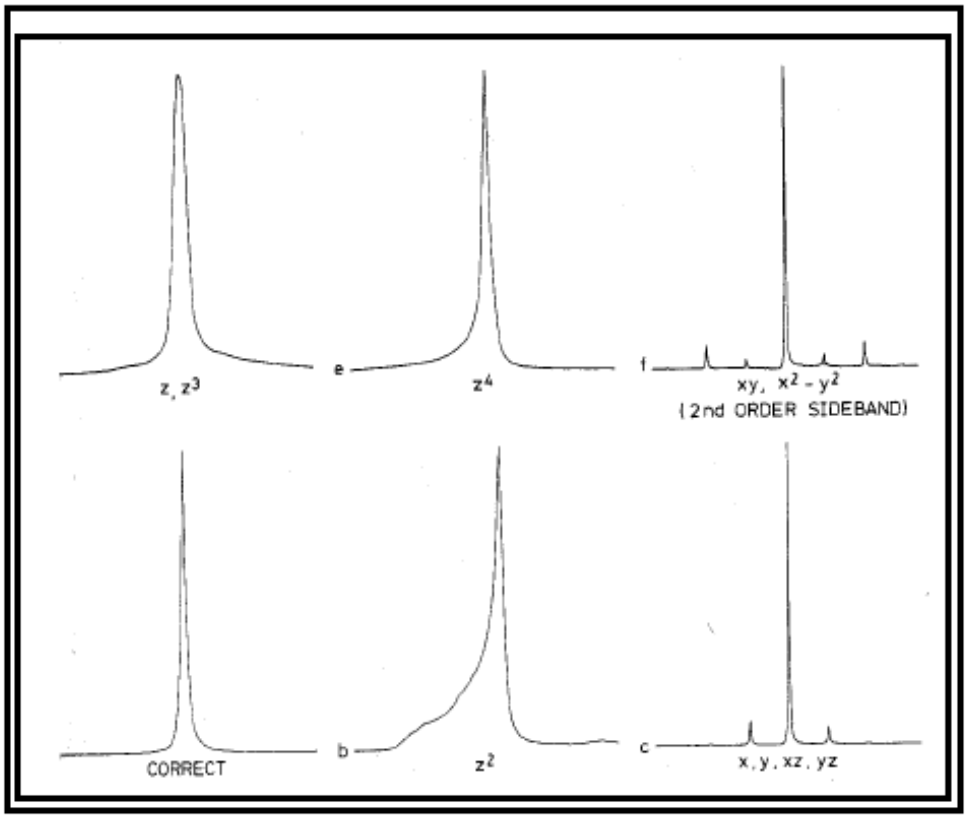


FIGURE 5. Misadjusted shims and appearance of corresponding signals after FT.

Another way of controlling the homogeneity of the magnetic field is to watch the shape of the FID. When the field is highly homogenous, the FID should fall smoothly following an exponential. The resolution of the signal determines how long the FID lasts:

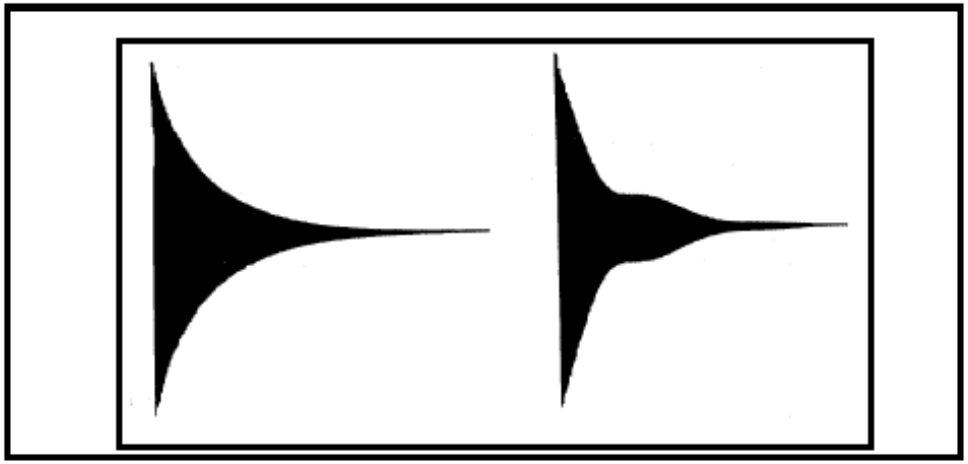
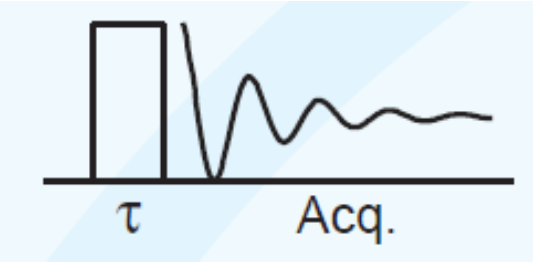
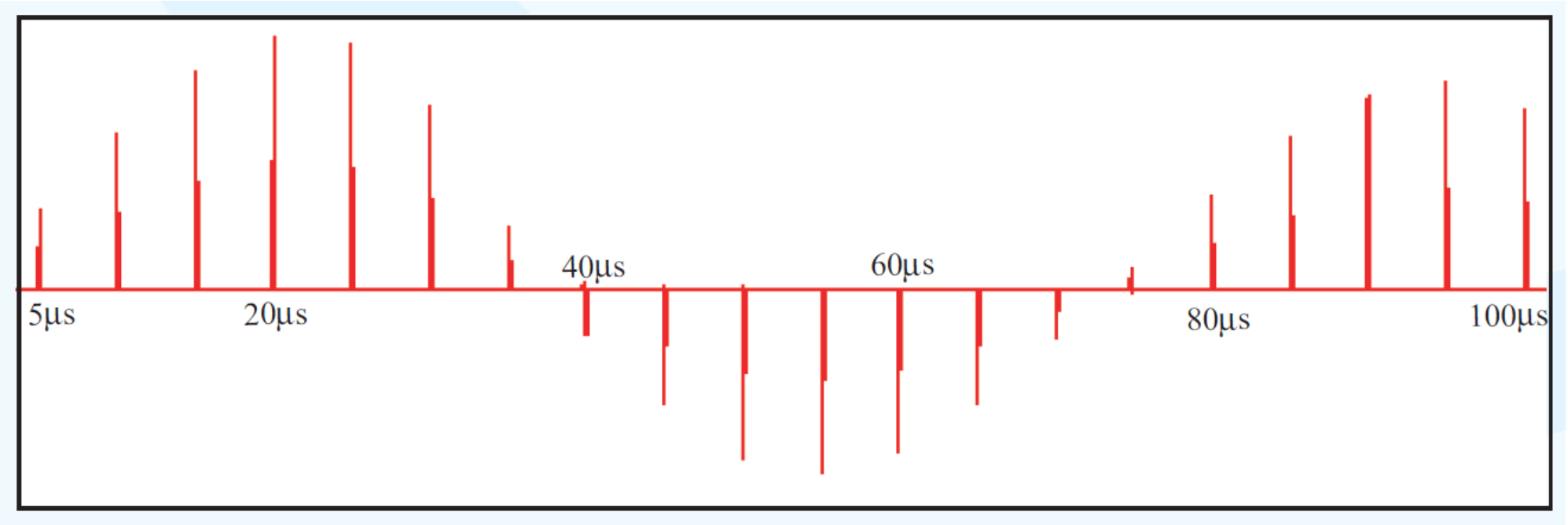


FIGURE 6. FID of perfectly shimmed magnet (left) and mis-shimmed magnet (right).

Before acquisition:
Pulse length calibration



$$\theta = \gamma B_1 \tau_p$$



A set of 1H spectra of H2O acquired using different pulse widths.

Process the data as 1D. Adjust the phase for pure absorption. Use the same phase correction for the rest of the records.

Spectrum processing: window multiplication

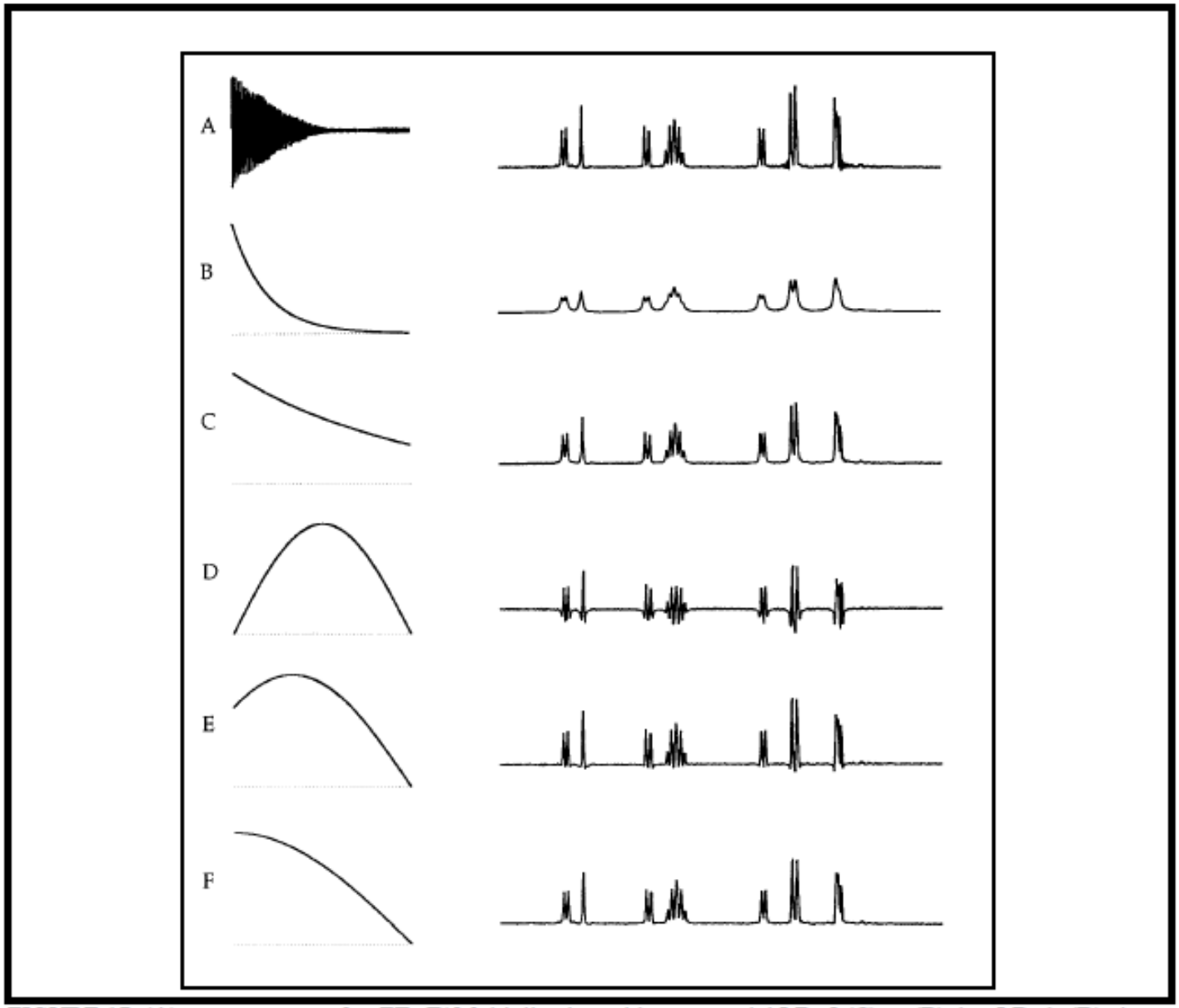


FIGURE 17. (A) raw spectrum after FT. (B)Multiplication with exponential,LB=5.(C) as (B), but LB=1. (D) sine-bell.(E) 45 degree shifted sine-bell.(F)90 degree shifted sine-bell.

Peak shape: absorption/dispersion modes

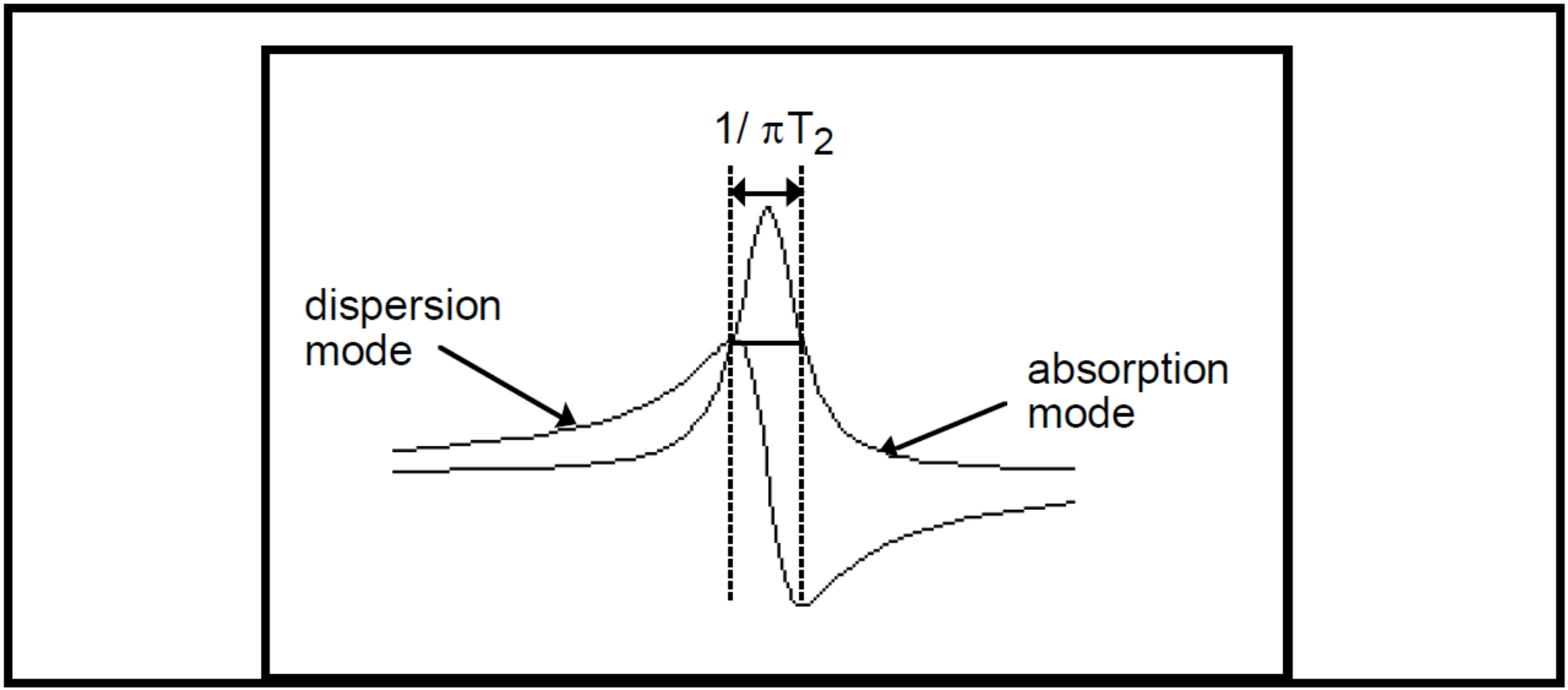


FIGURE 9. Signal in pure absorption or dispersion mode

Purely absorptive signals have a much narrower base, so that differentiation of peaks very close to each other is easier if they have been phased to yield purely absorptive line shape. Therefore, a phase-correction ϕ is needed to yield purely absorptive signals.

Spectrum processing
Phase modulation

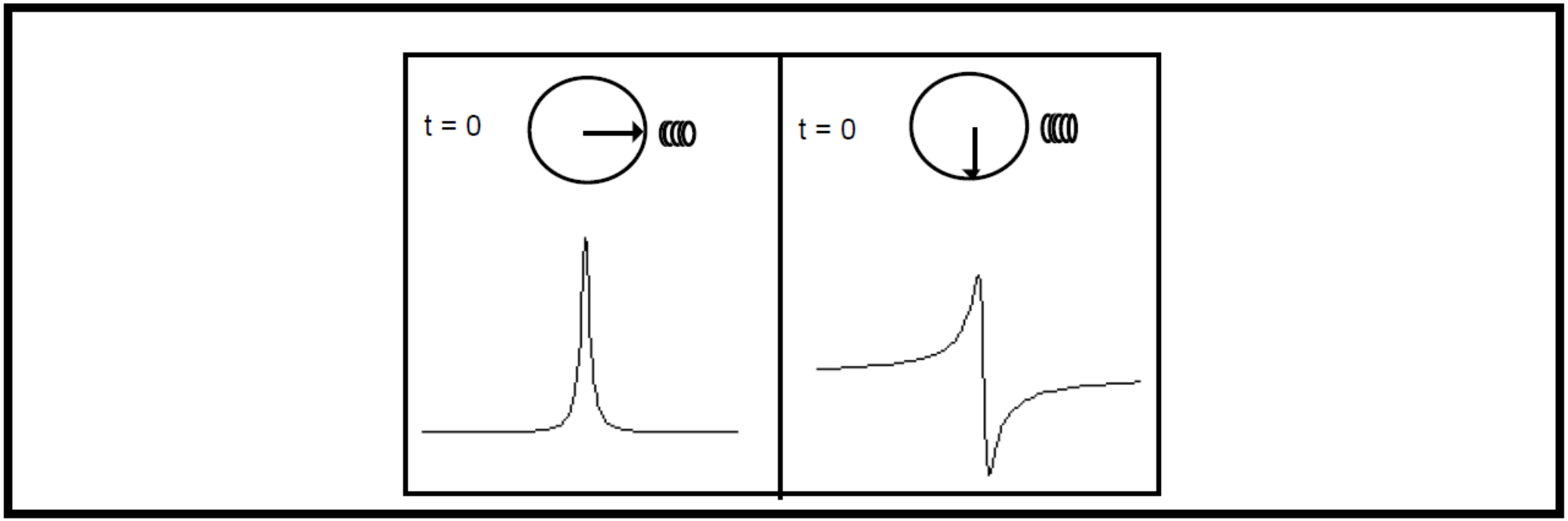


FIGURE 10. The relative phase of the magnetization with respect to the receiver coil determines the phase of the signal after Fourier transformation

What is the origin of the varying phase in the spectra? The zero-order arises because the absolute phase of the signals at the detector depends on the cable lengths etc. The linear dependence of the phase of the signals has a more complicated reason. In a theoretical 1D experiment, acquisition of the FID would start immediately after application of the pulse. However, in a real experiment there is a protection delay (called **de** on Bruker instruments), to wait for the pulse ring-down (pulsing and detection of the signal is done on the same coil, so that there must be a protection delay after the pulse):

Phase correction

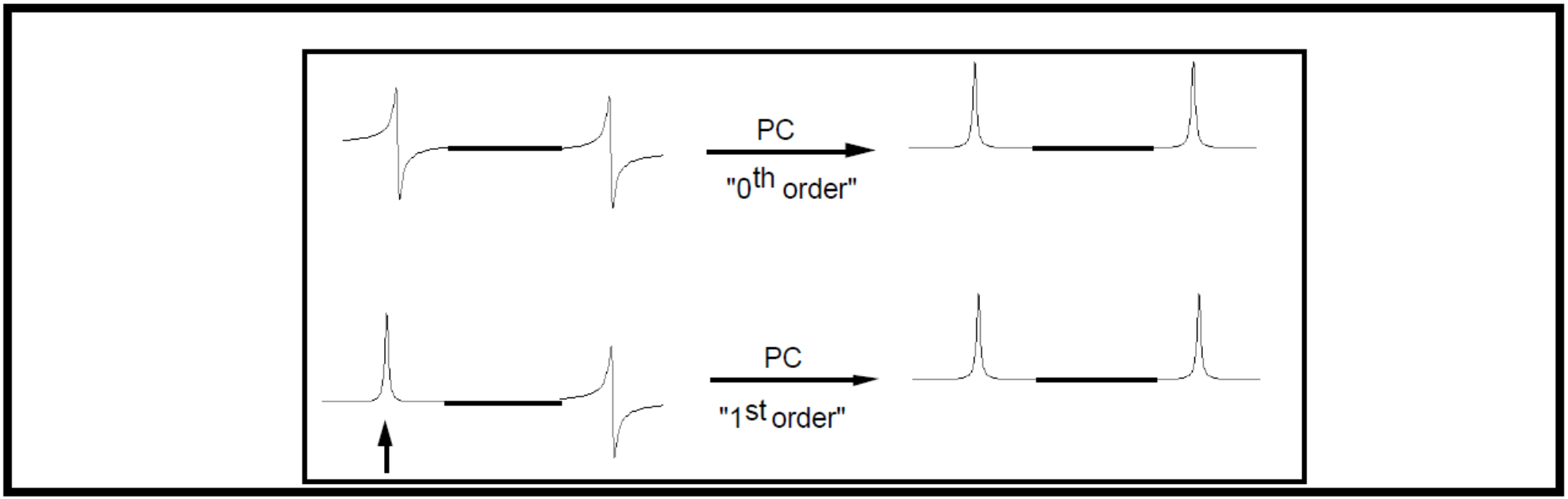


FIGURE 11. Top: The zero order phase correction changes the signal phase for all signals by the same amount. Lower: For the first order phase correction the applied correction depends on the frequency difference to the reference signal (marked by an arrow).

To adjust the phase, go into the phase correction mode, define a signal at one end of the spectrum as the reference phase, use the zero-order phase correction to phase it to absorption and then use the first order phase-correction to phase the signal at the other edge of the spectrum.

Baseline correction, receiver overflow

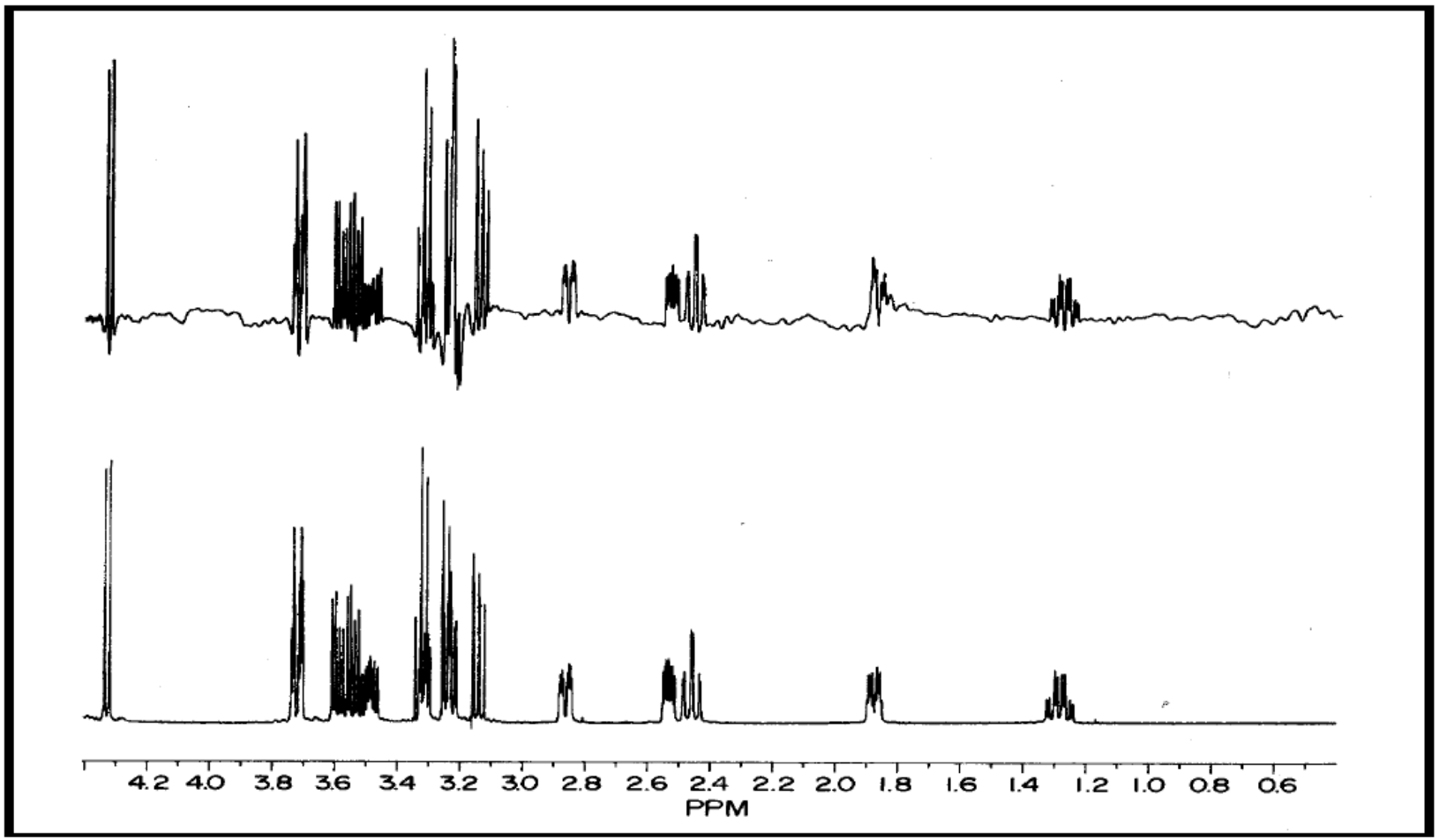


FIGURE 21. lower trace: spectrum after FT of an FID with correctly adjusted receiver gain. Top: Spectrum after FT of a clipped FID.