NMR の原理と活用方法（講義）
Principle and Application of Nuclear Magnetic Resonance Spectroscopy (Lecture in Japanese)

森 康友紀・古荘 義雄（生命科学講座・化学）
Yasuyuki Mori, Yoshio Furusho (Division of Chemistry)

核磁気共鳴分光法は有機化合物の構造を同定するための重要な測定法のひとつであり、有機化合物の水素や炭素などの化学的環境をはじめとした様々な情報を知ることができる。本法では、分子量が数十程度の低分子をはじめ、数万以上の高分子化合物まで適用できる。一般的な測定では、試料と重水素化された溶媒からなる溶液を用いるが、不溶性化合物の測定も可能であり、幅広い有機化合物の構造を同定できる。本講義では、核磁気共鳴分光法の原理と活用方法について説明するとともに、実際に有機化合物を測定する。

Nuclear magnetic resonance spectroscopy is one of the most important measurement methods for identifying the structure of organic compounds, providing information such as the chemical environment of hydrogen, carbon, and so on in organic compounds. This method can be applied to high molecular weight compounds like polymers and compounds with low molecular weights. In general, a solution consisting of an organic compound and a deuterated solvent is used in the measurement. Moreover, insoluble compounds can also be measured. Nuclear magnetic resonance spectroscopy enables the identification of the structure of a wide range of organic compounds. In this lecture, I will explain the principle and application of nuclear magnetic resonance spectroscopy. Additionally, we will use nuclear magnetic resonance spectroscopy for the measurement of organic molecules.

Principle and Application of Nuclear Magnetic Resonance Spectroscopy

Department of Fundamental Biosciences
Division of Chemistry
Yasuyuki Mori・Yoshio Furusho

Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a non-destructive analytical method for structural analysis of mainly organic compounds. NMR uses the property of atomic nuclei to resonate in a strong magnetic field when radio waves are applied externally.
MRI uses the same principle.

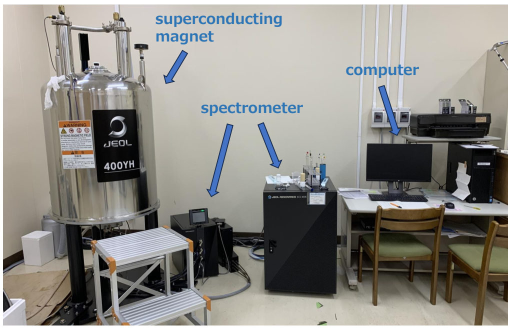
NMR

MRI

滋賀医科大学実験実習支援センターHPより
https://wwwcrl.shiga-med.ac.jp/home/kiki_bumon/g_book/mri/3t/home.html

Nuclear Magnetic Resonance Spectrometer

【NMR①（400 MHz） in SUMS】



For liquid sample

Nuclear Magnetic Resonance Spectrometer

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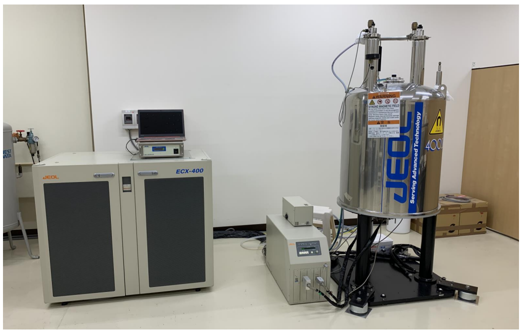


For solid sample

Classical Analysis of Molecular Structure

Ex : Structural analysis of organic compounds

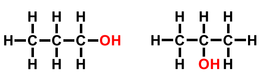
Pure sample ⇨ Confirming component element ⇨ Elemental analysis ⇨ Determining composition formula

⇨ Measuring molecular weight ⇨ Determining molecular formula ⇨ Confirming functional group

⇨ Distinguishing isomers ⇨ Determining structural formula

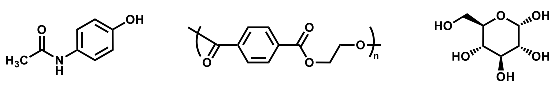
multi processes and long time

Using NMR, it is possible to estimate the type and number of hydrogen, carbon, and other atoms in a molecule.
single process and short time



Even if isomers, the structural formula can be easily estimated.

Application Area

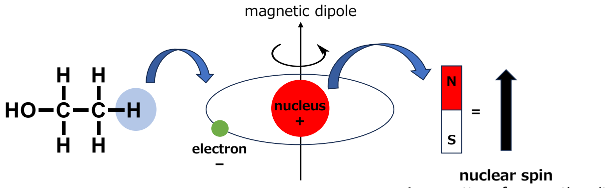


▶Organic compounds: Pharmaceuticals, natural products…
▶Polymeric materials: polyethylene, polyester…
▶Biomolecules: nucleic acid, protein…
▶Inorganic compounds

What we can know
▶Molecular structure
▶Reaction efficiency
▶Reaction rates
▶Interactions such as hydrogen bonding interactions
▶Diffusion coefficient etc

Principle of Nuclear Magnetic Resonance

Molecules consist of atoms, which are composed of a nucleus and electrons.
The nucleus has a positive charge and rotates, generating magnetic field.
In other words, an atom can be regarded as a small magnet.
This is called nuclear spin.



As a matter of convention, it is denoted by an arrow.

Measurable Nucleus

The presence of nuclear spin is necessary for NMR phenomena.
The presence or absence of nuclear spin can be determined by a physical quantity called spin quantum number. This value depends on the number of protons and neutrons in the nucleus.
If the number of protons and neutrons are both even, the spin quantum number is zero, and therefore does not exhibit NMR phenomena. Other nuclei can be measured.

表 3.1　有機化合物の NMR スペクトルに関連する核の性質

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^{10}B	3	2.875	1.801 b)	19.6	0.020	$3.90 \cdot 10^{-3}$	10.747
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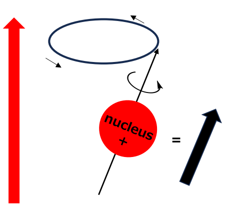
spin quantum number

野村正勝ら，有機化学のためのスペクトル解析法（第2版），化学同人，2010，74.

Principle of Nuclear Magnetic Resonance

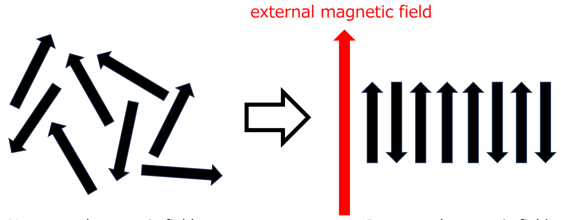
In a magnetic field, nucleus rotates like a collapsing top (**Larmor precession**).
The frequency of this rotation is equal to the resonance frequency.



Larmor precession

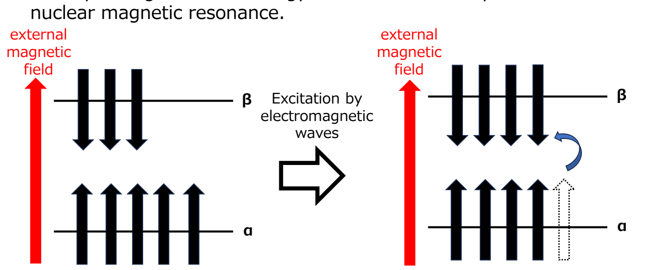
Principle of Nuclear Magnetic Resonance

Since nuclei normally rotate in random directions, the orientation of nuclear spins is also random.
When a strong external magnetic field is applied, the nuclear spins align in two different ways, either in forward or reverse parallel to the magnetic field.



Principle of Nuclear Magnetic Resonance

Nuclear spins in inverse parallel oppose the external magnetic field and are more energetic than those in forward parallel.
The splitting of nuclear spins into two energy levels under the influence of an external magnetic field is called **Zeeman splitting**.
In this state, the system resonates with electromagnetic waves corresponding to the energy difference. This phenomenon is nuclear magnetic resonance.

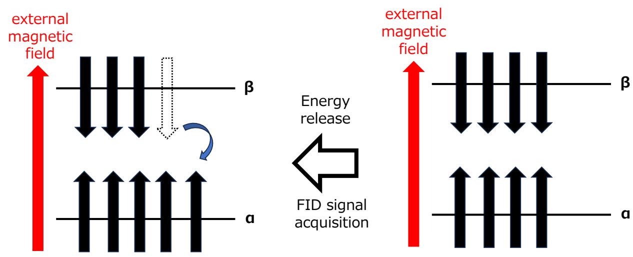


State classified by energy (β>α)
Zeeman splitting

Nuclear spin shifts to higher energy states
nuclear magnetic resonance

Principle of Nuclear Magnetic Resonance

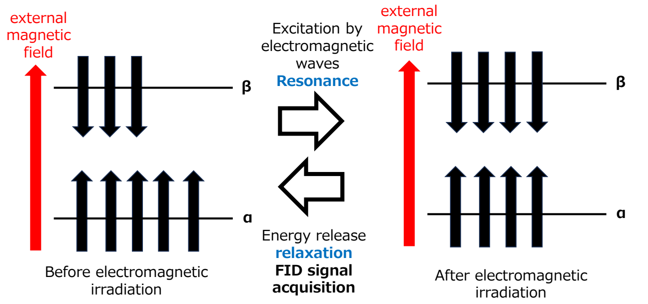
Excited nuclear spins return to their initial state in the absence of electromagnetic radiation. This phenomenon is called relaxation.
By detecting the energy released during relaxation, a **free inductive decay (FID) signal** is obtained.



Nuclear spin shifts to lower energy states
relaxation

Nuclear spins after nuclear magnetic resonance

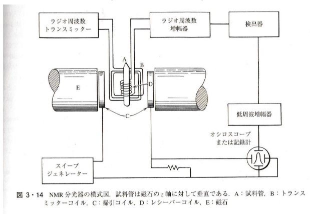
Principle of Nuclear Magnetic Resonance



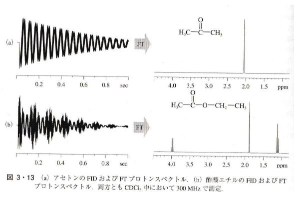
Principle of Nuclear Magnetic Resonance

When a magnetic field is applied to a sample and radio waves are emitted by a radio frequency oscillator, nuclear magnetic resonance occurs. At this time, a minute induced current is emitted in the coil surrounding the sample, which is recorded as a free inductive decay (FID) signal via an amplifier.
By Fourier transforming this FID signal, an NMR spectrum can be obtained (time is divided into signals of each component and converted to frequency).

【Schematic diagram of NMR spectrometer】



【FID and FT proton spectra】



荒木峻ら，有機化合物のスペクトルによる同定法（第7版）,東京化学同人，2006，140-141.

Information obtained by NMR

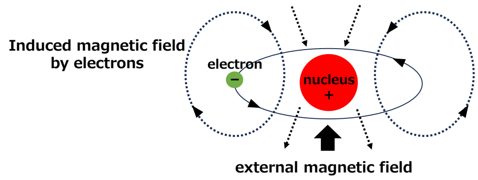
Chemical shift (signal position):
Information about the chemical environment of nuclei (e.g., functional groups)

Spin-spin coupling (signal splitting):
Information about the relationship with neighboring nuclei

Signal intensity (area of the signal):
Information about the ratio of the number of nuclei.

Chemical shift

The electrons around a nucleus circulate in a magnetic field and create an induced magnetic field to weaken the external magnetic field (= shielding effect). This weakening effect changes according to the chemical environment, i.e., nucleus location in the chemical structure.

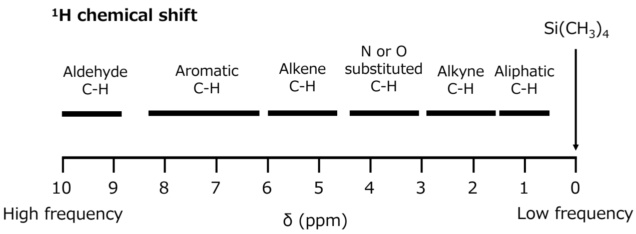


Tetramethylsilane [TMS, $Si(CH_3)_4$] is often used as a standard compound in ^{1}H or ^{13}C NMR spectra.
The TMS signal is taken as 0 ppm.

δ（ppm） = Resonance frequency of a certain nuclide（Hz）/Spectrometer frequency（MHz）

Chemical shift

Magnitude of chemical shift
In general, the chemical shift δ is a few Hz~hundreds of Hz, and is very small compared to the resonance frequency of several hundred MHz.
→Expressed in ppm relative to the resonance frequency (ppm: parts per million).
The larger resonance frequency, the larger the number.

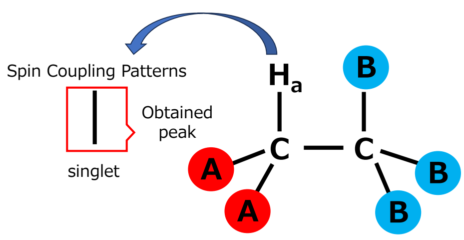


Spin-spin coupling

Information about the relationship with neighboring nuclei.

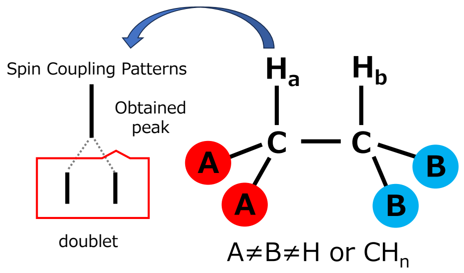
In the case of ^{1}H

Protons through chemically bonded electrons spin-interact.
As a result, the peak splits.

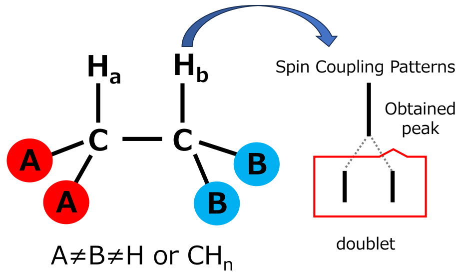


A≠B≠H or CH_n

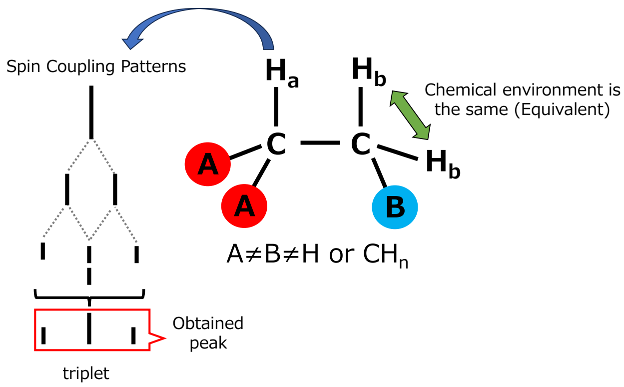
Spin-spin coupling



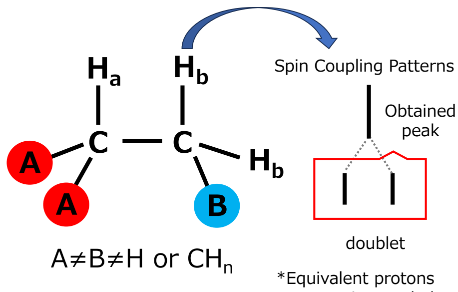
Spin-spin coupling



Spin-spin coupling

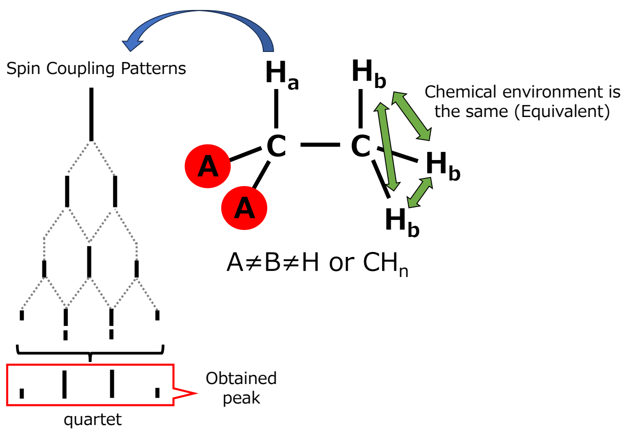


Spin-spin coupling

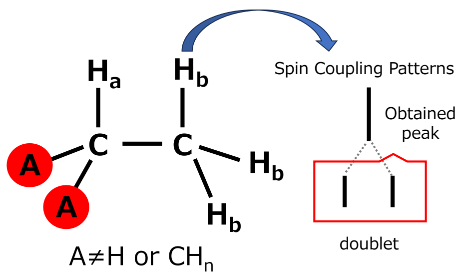


*Equivalent protons are not spin-coupled.

Spin-spin coupling



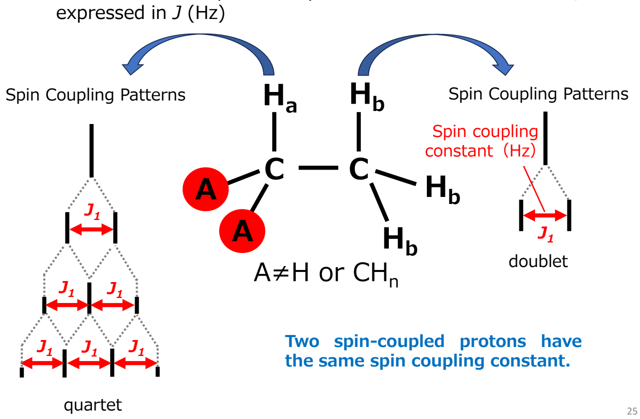
Spin-spin coupling



When there are n protons on the carbon adjacent to a given proton, the number of peaks in the multiline is n+1.

Spin coupling constant

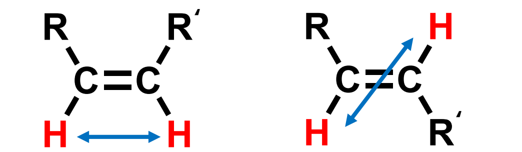
The width of the split of the peak that becomes a multiline, expressed in J (Hz)



Two spin-coupled protons have the same spin coupling constant.

Prediction of stereochemistry based on spin coupling constants

The structure-specific spin coupling constants are known.
This is very useful for structure determination.



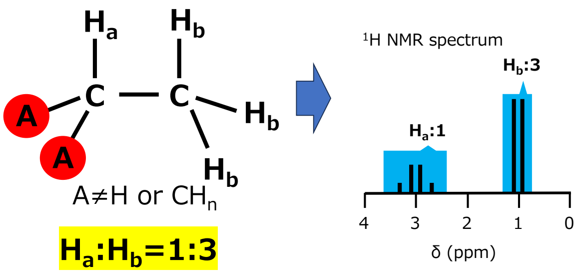
J = approximately 10 Hz — **cis** form

J = approximately 17 Hz — **trans** form

Signal intensity (area of the signal)

Information about the ratio of the number of nuclei.

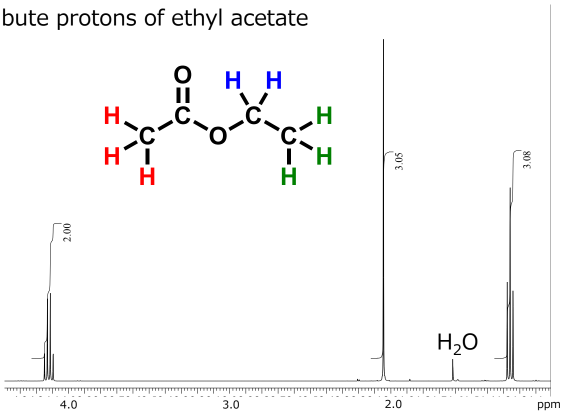
The area of each peak (by integration) is directly proportional to the number of protons belonging to that peak.



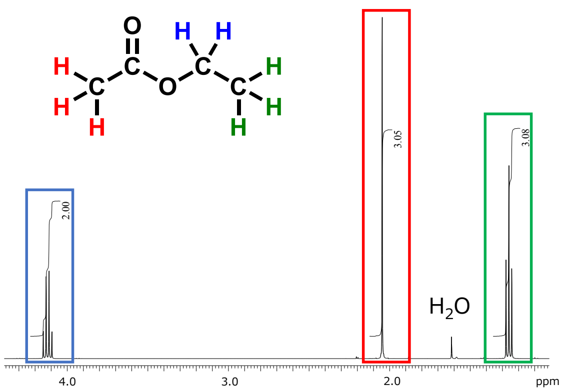
H_a:H_b=1:3

Quiz

Attribute protons of ethyl acetate

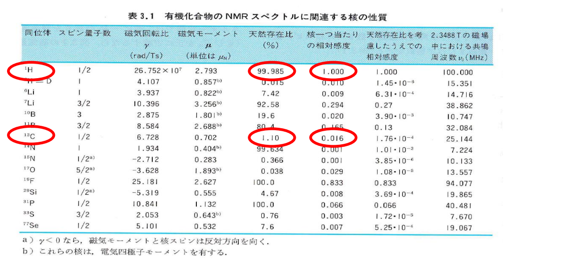


Answer



Characteristics of ^{13}C NMR measurements

Since the natural existence ratio is small and the sensitivity is weak, a large amount of sample and a long measurement time are required for the measurement.
※^{12}C has a spin quantum number of 0, so NMR measurement is not possible.



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▶Since the natural existence ratio is small and the sensitivity is weak, a large amount of sample and a long measurement time are required for the measurement.
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▶ Peak intensity is usually not related to the number of carbon atoms.

▶ They are distributed over a wider range of chemical shifts than protons (0-220 ppm).

▶ Typically, removing the spin coupling to the proton Compound pulse decoupling method is used.
→ Basically, the peak is a single line.

Influence of spin coupling with ^{1}H in ^{13}C NMR measurements

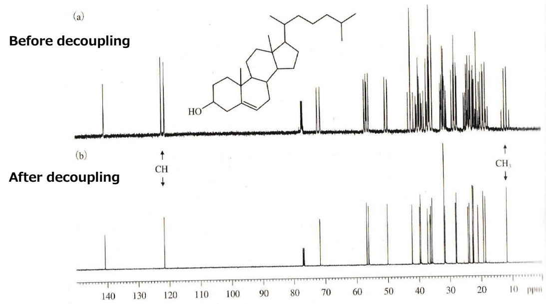


図 4・1 (a) コレステロールのプロトンとスピン結合した ^{13}C スペクトル．(b) コレステロールのプロトンをデカップルした ^{13}C スペクトル．両方とも 150.9 MHz において $CDCl_3$ 中で測定．

Before decoupling, the peaks are complex and difficult to analyze.

野村正勝ら，有機化学のためのスペクトル解析法（第2版），化学同人，2010，210.

2D NMR

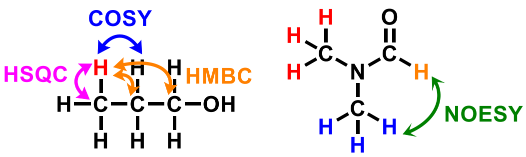
Typical example

^{1}H-^{1}H COSY (correlation spectroscopy)
A measurement to obtain correlations between all hydrogens connected via spin coupling.

^{1}H-^{13}C HSQC (heteronuclear single quantum correlation)
A measurement to obtain direct H-C bond correlations.

^{1}H-^{13}C HMBC (heteronuclear multiple bond correlation)
A measurement to obtain correlations between 2~3 H-C bonds.

NOESY (Nuclear Overhauser Effect Spectroscopy)
A measurement that detects hydrogen atoms in close proximity using an interaction called NOE that occurs between nuclei in close spatial proximity.



Measurement Method

①Sample preparation

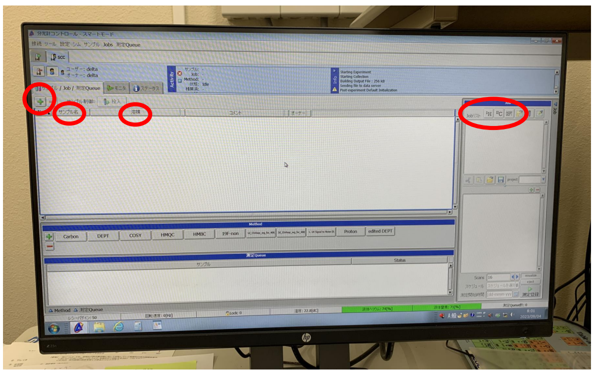
②Insertion into rotor

Measurement Method

③Place the rotor in the magnet.
The rotor is floating because of the air from the magnet.

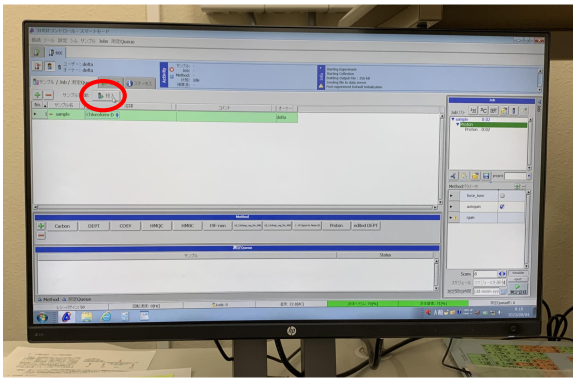
Measurement Method

④ Operation on PC: press "+" and input measurement information.



Measurement Method

⑤Operation on PC: press “投入".

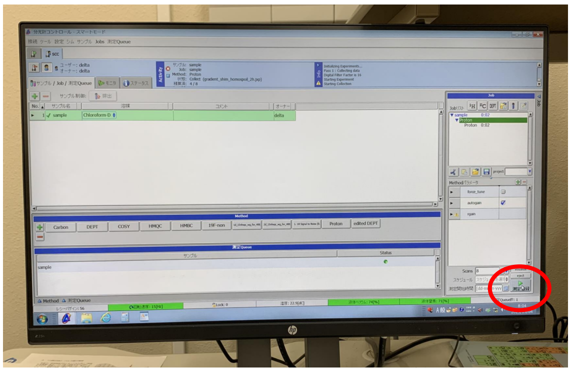


Measurement Method

⑥After pressing “投入" on the PC, the sample enters the magnet.

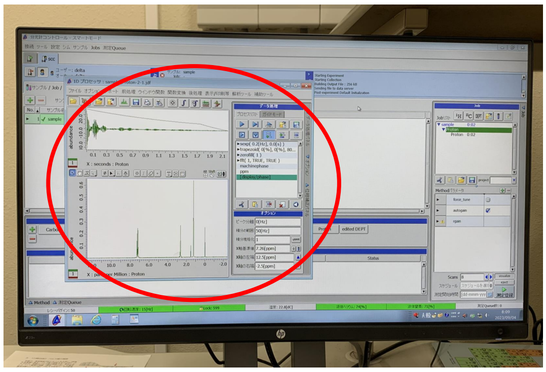
Measurement Method

⑦Press “測定登録" to start measurement.



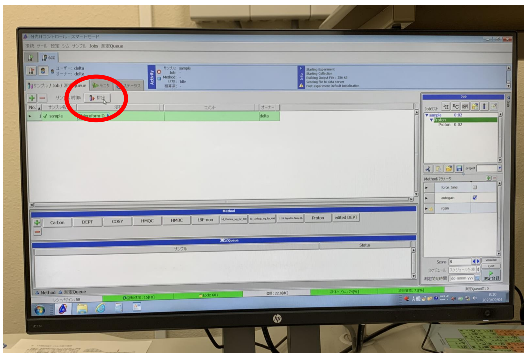
Measurement Method

⑧In the case of ^{1}H, the measurement takes 5 minutes (8 scans).
Then, the spectrum is obtained.



Measurement Method

⑨Press “排出" to take out the sample.

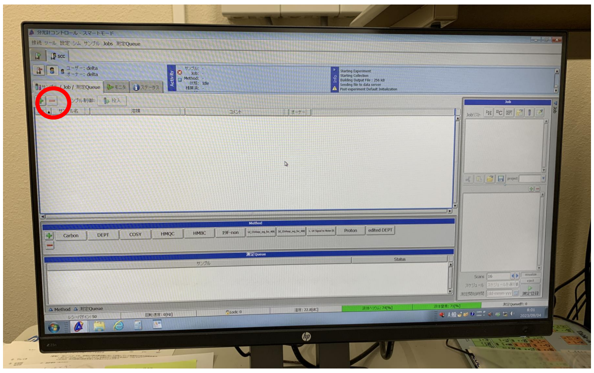


Measurement Method

⑩Extract the sample

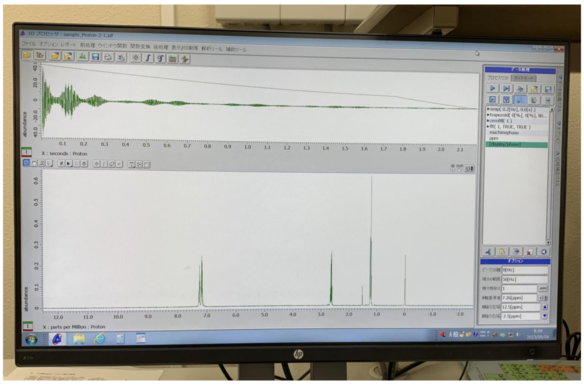
Measurement Method

⑪Delete information by pressing "-" on the PC



Measurement Method

⑫Analysis of spectra (data is automatically saved)



Reagent and Equipment for NMR

・Sample (5-10 mg)
In the case of ^{13}C, 20-30 mg of samples are suitable.

・Deuterated solvent (0.5-0.6 mL)
$CDCl_3$, CD_3OD, D_2O, etc.

・Glass tube for NMR (with cap)

*Spectral Database for Organic Compounds (free)
https://sdbs.db.aist.go.jp/