核磁気共鳴分光法と質量分析法の原理と活用方法（講義）
Principle and application of nuclear magnetic resonance spectroscopy and mass spectrometry
（Lecture in Japanese）

森 康友紀（生命科学講座・化学）
Yasuyuki Mori（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といっ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。質量分析法は有機化合物等の分子量を調べるための測定法である。適切な測定条件を用いることにより、分子量が数十程度の低分子や数千程度のタンパク質および数万以上の高分子化合物の分子量を測定できる。本講義では、核磁気共鳴分光法と質量分析法の原理と活用方法について説明する。

Nuclear magnetic resonance spectroscopy is one of the most important measurement methods for identifying the structure of organic compounds, providing various 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 as well as 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. Mass spectrometry is a measurement method to determine the molecular weight of organic compounds. By using appropriate measurement conditions, it is possible to measure the molecular weights of organic compounds with from low to high molecular weights. In this lecture, the principles and application of nuclear magnetic resonance spectroscopy and mass spectrometry will be explained.

Principle and Application of Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry

Yasuyuki Mori (Division of Chemistry)

Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is an analytical method for structural analysis of organic compounds composed of atoms such as carbon, hydrogen, oxygen, nitrogen, and phosphorus.
It uses the property of atomic nuclei to resonate in a strong magnetic field when radio waves are applied externally. (MRI uses the same principle).

Application area
▶Low to medium molecular weight organic compounds: Pharmaceuticals, agrochemicals, natural products
▶Polymeric materials: polyethylene, polyester
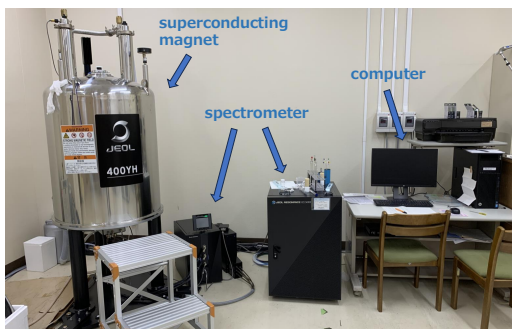▶Biomolecular materials: nucleic acids, proteins

What we know
▶Molecular structure
▶Reaction efficiency
▶Reaction ratesInteractions such as hydrogen bonding interactions
▶Diffusion coefficient

Nuclear Magnetic Resonance Spectrometer
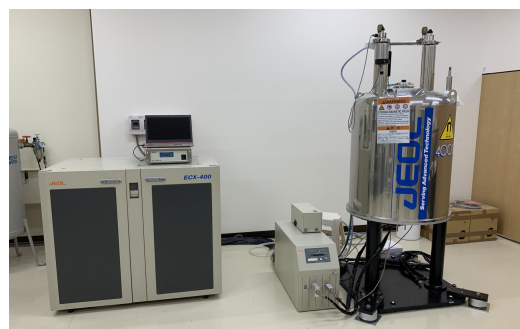
【NMR①（400 MHz） in SUMS】

For liquid samples

Nuclear Magnetic Resonance Spectrometer

【NMR②（400 MHz） in SUMS】

For solid samples

Principle of Nuclear Magnetic Resonance

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

nuclear spin
（As a matter of convention, it is denoted by an arrow.）
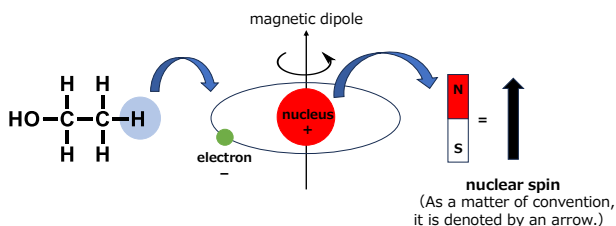
Measurable Nucleus

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 nucleus 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

| 同位体	スピン量子数	磁気回転比 γ (rad/Ts)	磁気モーメント μ (単位は μN)	天然存在比 (%)	核一つ当たりの相対感度	天然存在比を考慮したうえでの相対感度	2.3488Tの磁場中における共鳴周波数 ν (MHz)
¹H	1/2	26.752×10⁷	2.793	99.985	1.000	1.000	100.000
²H≡D	1	4.107	0.857⁽ᵃ⁾	0.015	0.010	1.45・10⁻⁶	15.351
⁶Li	1	3.937	0.822⁽ᵃ⁾	7.42	0.009	6.31・10⁻⁴	14.716
⁷Li	3/2	10.396	3.256⁽ᵃ⁾	92.58	0.294	0.27	38.862
¹⁰B	3	2.875	1.801⁽ᵃ⁾	19.6	0.020	3.90・10⁻³	10.747
¹¹B	3/2	8.584	2.688⁽ᵇ⁾	80.4	0.165	0.13	32.084
¹³C	1/2	6.728	0.702	1.10	0.016	1.76・10⁻⁴	25.144
¹⁴N	1	1.934	0.404⁽ᵇ⁾	99.634	0.001	1.01・10⁻³	7.224
¹⁵N	1/2⁽ᵃ⁾	-2.712	0.283	0.366	0.001	3.85・10⁻⁶	10.133
¹⁷O	5/2⁽ᵃ⁾	-3.628	1.893⁽ᵇ⁾	0.038	0.029	1.08・10⁻⁵	13.557
¹⁹F	1/2	25.181	2.627	100.0	0.833	0.833	94.077
²⁹Si	1/2⁽ᵃ⁾	-5.319	0.555	4.67	0.008	3.69・10⁻⁴	19.865
³¹P	1/2	10.841	1.132	100.0	0.066	0.066	40.481
³³S	3/2	2.053	0.643⁽ᵇ⁾	0.76	0.003	1.72・10⁻⁵	7.670
⁷⁷Se	1/2	5.101	0.532	7.6	0.007	5.25・10⁻⁴	19.067

a) γ＜0なら、磁気モーメントと核スピンは反対方向を向く。
b) これらの核は、電気四極子モーメントを有する。

spin quantum number

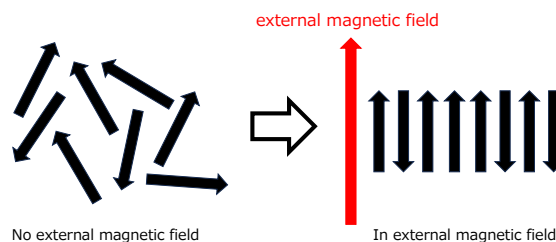## Principle of Nuclear Magnetic Resonance

In a magnetic field, nucleus rotate like a collapsing top (Larmor precession).
The frequency of this rotation is equal to the resonance frequency.
Example: In 400 MHz NMR, ^{1}H is 400 MHz.

external magnetic field
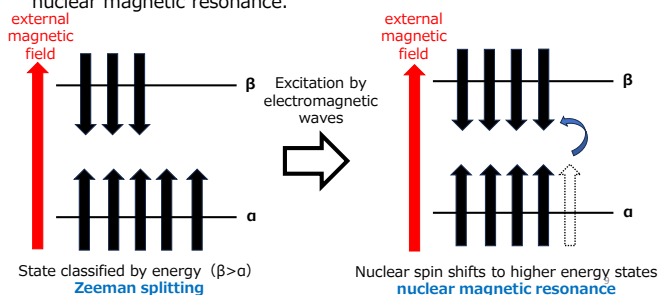
nucleus +

=

Larmor precession
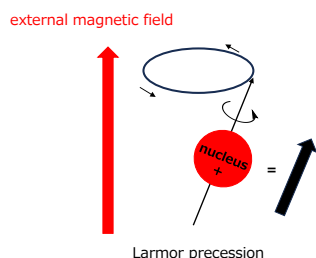
7

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.

external magnetic field

No external magnetic field
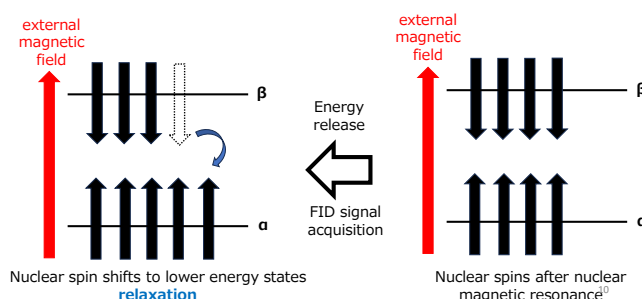
In external magnetic field

8

Principle of Nuclear Magnetic Resonance

Nuclear spins in inverse parallel oppose the external magnetic field and are therefore 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.

external magnetic field

β

Excitation by electromagnetic waves

external magnetic field

β

α

α

State classified by energy （β>α)
Zeeman splitting

Nuclear spin shifts to higher energy states
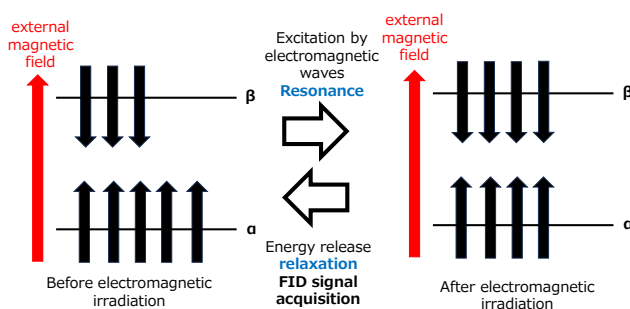**nuclear magnetic resonance**

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.

external magnetic field

β

Energy release

external magnetic field

β

α

FID signal acquisition

α

Nuclear spin shifts to lower energy states
relaxation

Nuclear spins after nuclear magnetic resonance

10

Principle of Nuclear Magnetic Resonance

external magnetic field

β

Excitation by electromagnetic waves
Resonance

external magnetic field

β

α

Energy release
relaxation
FID signal acquisition

α

Before electromagnetic irradiation

After electromagnetic irradiation

11

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).
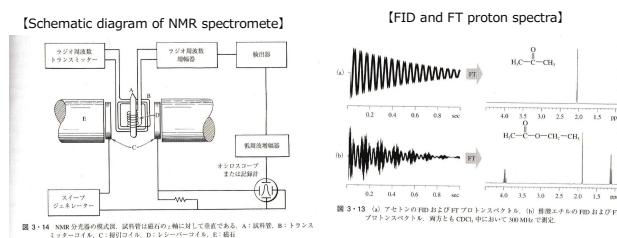
【Schematic diagram of NMR spectromete】

【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 on the chemical structure of nuclei (e.g., functional groups) Information about the chemical structure 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.

13

Chemical shift

The resonance frequency of a nucleus depends on the chemical environment in which it is located(due to the effect of the induced magnetic field by electrons = shielding effect).The position of the signal is indicated by its relative position to the standard compound, and this deviation is called the chemical shift.

Induced magnetic field by electrons

electron

nucleus +

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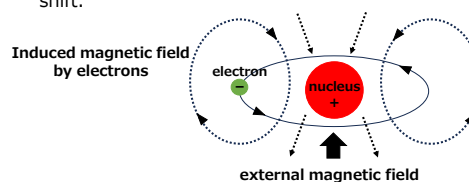
Tetramethylsilane [TMS, $Si(CH_3)_4$] is often used as a standard compound in ^1H or ^{13}C NMR spectra.
The TMS signal is taken as 0 ppm.

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

14

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).
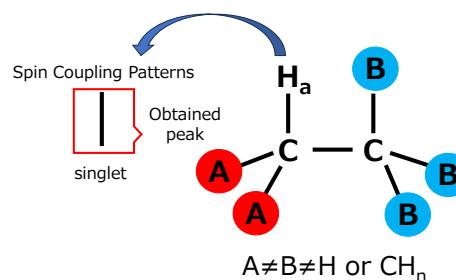The larger the resonance frequency, the larger the number.

^1H chemical shift

$Si(CH_3)_4$

| Aldehyde C-H | Aromatic C-H | Alkene C-H | N or O substituted C-H | Alkyne C-H | Aliphatic C-H |

10　9　8　7　6　5　4　3　2　1　0

High frequency   δ (ppm)   Low frequency
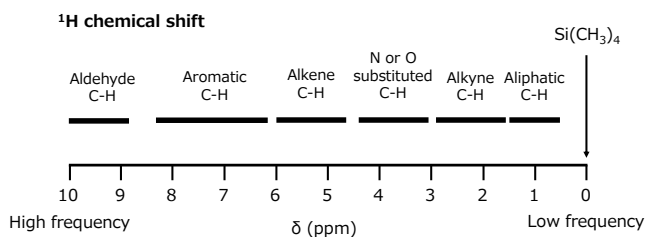
15

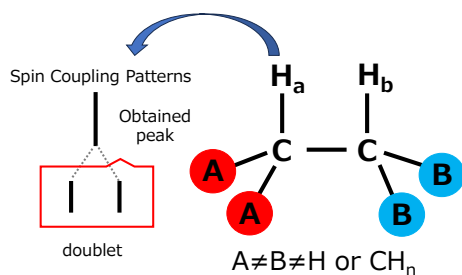## Spin-spin coupling

Information about the relationship with neighboring nuclei.

In the case of ^1H

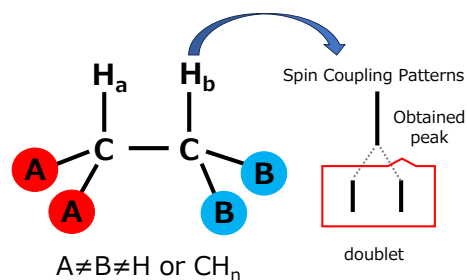Protons through chemically bonded electrons spin-interact.
As a result, the peak splits.

Spin Coupling Patterns

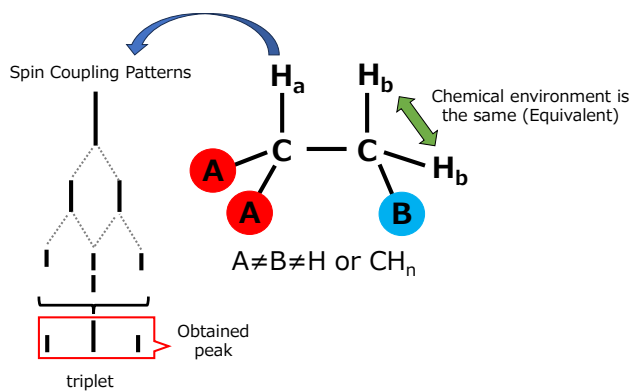Obtained peak

singlet

H_a

A A C C B B

B

$A{\neq}B{\neq}H$ or CH_n

16

Spin-spin coupling

Spin Coupling Patterns

Obtained peak

doublet

H_a H_b

A A C C B B

$A{\neq}B{\neq}H$ or CH_n

17

Spin-spin coupling

H_a H_b

A A C C B B

Spin Coupling Patterns

Obtained peak

doublet

$A{\neq}B{\neq}H$ or CH_n

18

Slide 19

Spin-spin coupling

Spin Coupling Patterns

H_a H_b

A C — C H_b

A B

Chemical environment is the same (Equivalent)

A≠B≠H or CH_n

Obtained peak

triplet

19

Slide 20

Spin-spin coupling

H_a H_b

Spin Coupling Patterns

A C — C H_b

A B

A≠B≠H or CH_n

Obtained peak

doublet

*Equivalent protons are not spin-coupled no spin coupling

20

Slide 21

Spin-spin coupling

Spin Coupling Patterns

H_a H_b

A C — C H_b

A H_b

Chemical environment is the same (Equivalent)

A≠B≠H or CH_n

Obtained peak

quartet

21

Slide 22

Spin-spin coupling

H_a H_b

Spin Coupling Patterns

A C — C H_b

A H_b

A≠H or CH_n

Obtained peak

doublet

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

22

Slide 23

Spin coupling constant

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

Spin Coupling Patterns

H_a H_b

Spin Coupling Patterns

A C — C H_b

A H_b

Spin coupling constant（Hz）

A≠H or CH_n

doublet

J_1
J_1 J_1
J_1 J_1 J_1

quartet

J_1

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

23

Slide 24
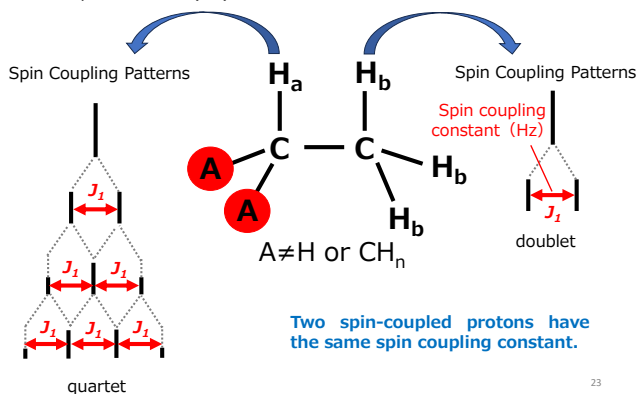
Prediction of stereochemistry based on spin coupling constants

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

R R′
C＝C
H H

J = approximately 10 Hz

cis form

R H
C＝C
H R′

J = approximately 17 Hz

trans form

R≠R′≠H or CH_n
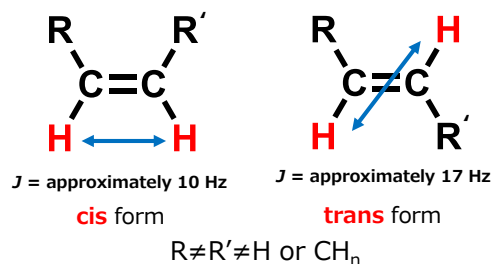
24

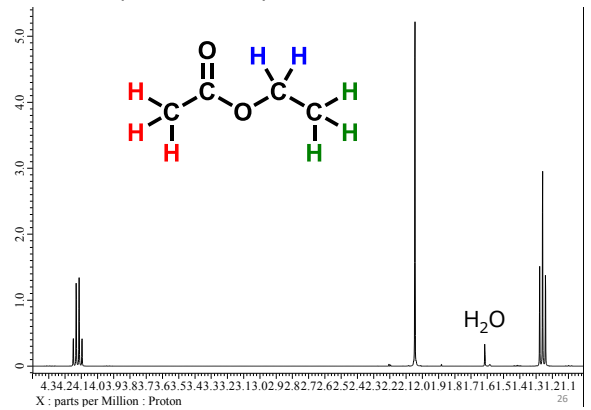## Signal intensity (area of the signal)

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.
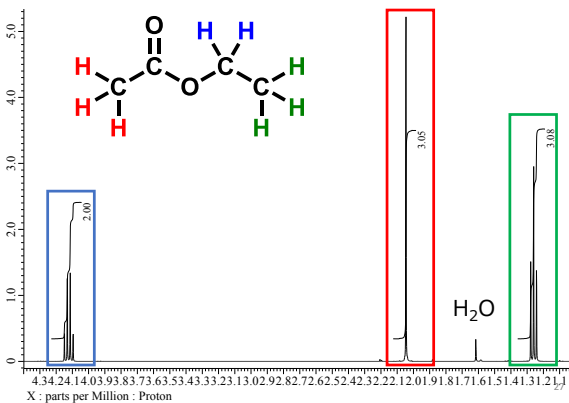
^{1}H NMR spectrum

H_a:1 H_b:3

δ (ppm)

$H_a:H_b=1:3$

A≠H or CH_n

Quiz

Attribute the proton of ethyl acetate.

H_2O

X : parts per Million : Proton

Answer

H_2O

X : parts per Million : Proton

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.

表 3.1 有機化合物の NMR スペクトルに関連する核の性質

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.

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.

▶ 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

(a)
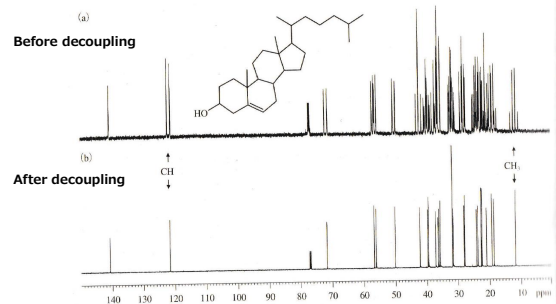Before decoupling

(b)
After decoupling

CH

CH_3

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.

Measurement Method

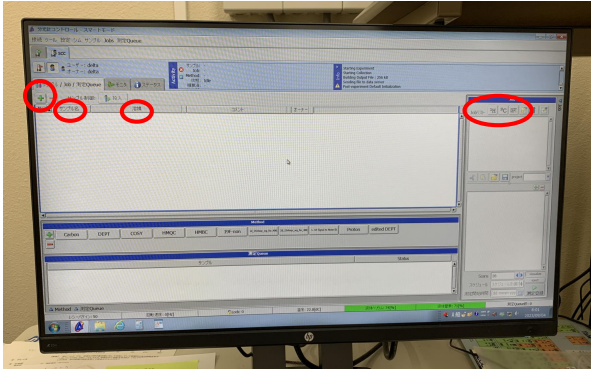①Sample preparation   ②Insertion into rotor

Measurement Method

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

Measurement Method
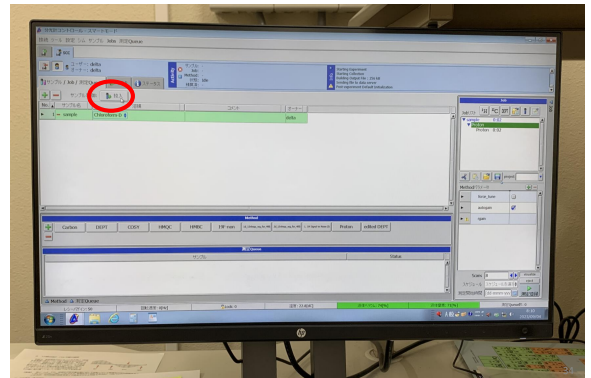
④ Operation on PC: Press "+" and input measurement information.

Measurement Method

⑤Operation on PC:
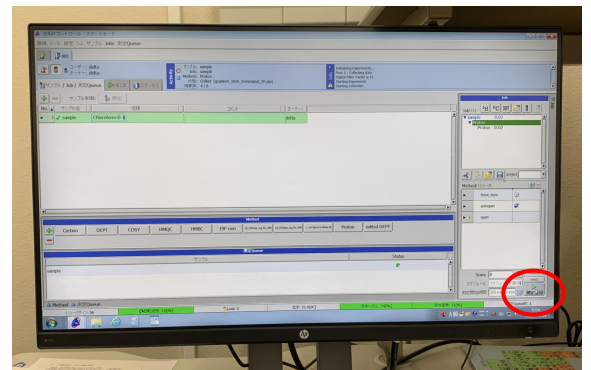Press "投入" after entering measurement information.

Measurement Method

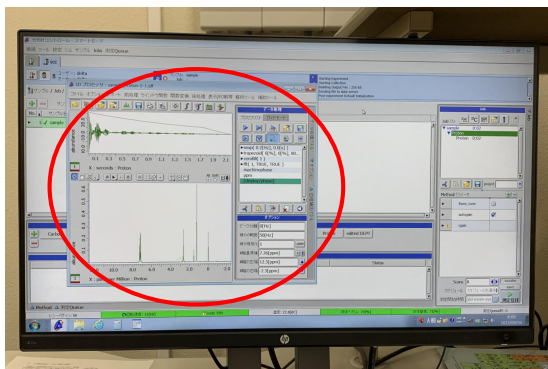⑥Pressing "投入" on the PC, the sample enters the magnet.

Measurement Method

⑦Press "測定登録" to start measurement.
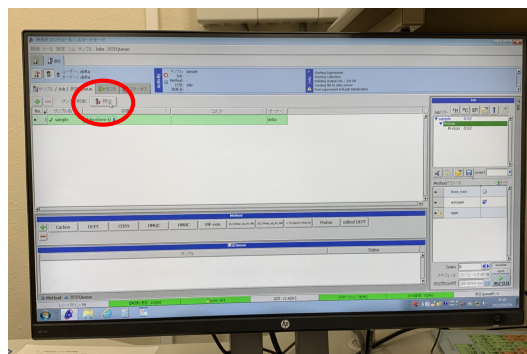
Measurement Method

⑧In the case of ^1H, the measurement is completed in about 5 minutes and the spectrum is obtained.

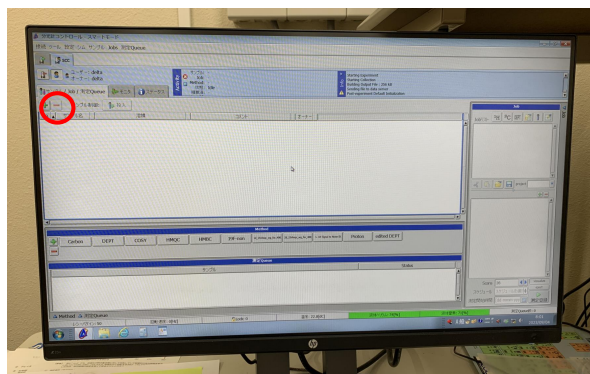## Measurement Method

⑨Press "排出" to take out the sample.
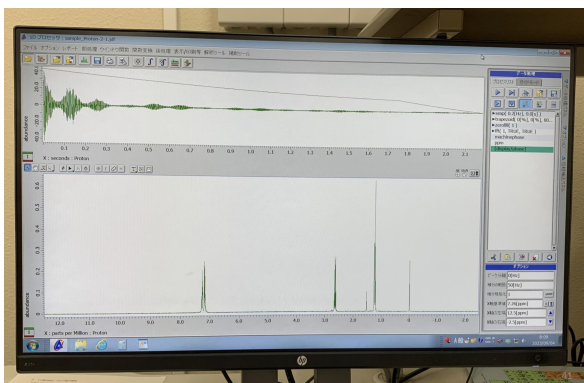

Measurement Method

⑩Extract the sample

Measurement Method

⑪Delete information by pressing "-" on the PC

Measurement Method

⑫Analysis of spectra (data is automatically saved)

What reagents and equipment are needed?

・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)

Mass Spectrometry

It is an analytical technique that ionizes atoms or molecules, accelerates them in a high vacuum, moves them through an electric or magnetic field, and separates and detects them using the difference in interaction with the field caused by the mass of each ion species.It can be measured using very small amounts of sample and is applicable to gaseous, liquid, and solid samples.

Application
▶ Low to medium molecular weight organic compounds:
Pharmaceuticals, agrochemicals, natural products
▶ Polymeric materials: polyethylene, polyester, and etc.
▶ Biomolecular materials: nucleic acids, proteins

What we know
▶Molecular weight
▶Atomic composition
▶Partial molecular structure

43

Mass Spectrometer Configuration

· Sample introduction section
· Ion generation section
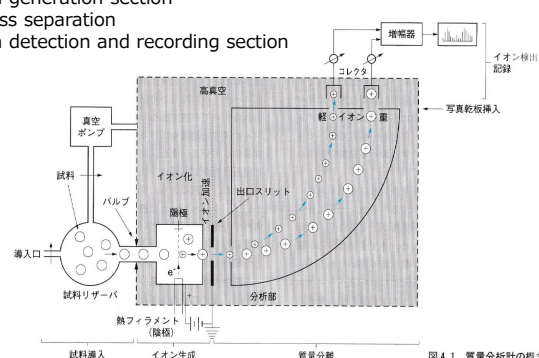· Mass separation
· Ion detection and recording section

図4.1 質量分析計の概念図

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, 238.

44

Types of ionization methods commonly used in mass spectrometry

Electron impact ionization (EI)

Chemical ionization (CI)

Fast Atomic Bombardment (FAB)

Electrospray ionization (ESI)

Thermospray ionization (TSI)

Atmospheric pressure ionization (API)

Matrix-assisted laser desorption/ionization (MALDI)

45

Type of Mass Separation Section

Magnetic field type

Quadrupole type

Ion trap type

Time-of-flight type

Fourier transform type

46

Mass spectrometer in SUMS

Gas chromatograph-mass spectrometer (GC-MS)
▶ Electron impact ionization method + quadrupole type
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOFMS)
▶ Matrix-assisted laser desorption/ionization + time-of-flight type
Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

MALDI-TOFMS

GC-MS

ICP-MS

Principle of Electron Impact Ionization

Molecules can be ionized by bombarding them with a beam of electrons.

$$M + e^- \longrightarrow M^{+\cdot} + 2e^-$$

M:molecule、e⁻:electron

In rare cases, divalent ions may be formed.

$$M + e^- \longrightarrow M^{2+} + 3e^-$$

48

Features of Electron Impact Ionization

・Detected Ions : M$^{+\cdot}$

・Approximate highest integer mass measurable : 3500

・The fragment ions provide structural information.

・Ionization requires vaporization of the sample.
(Possibility of thermal decomposition, samples that do not vaporize cannot be measured)

Ionization method effective for organic compounds of medium to low polarity and low molecular weight

49

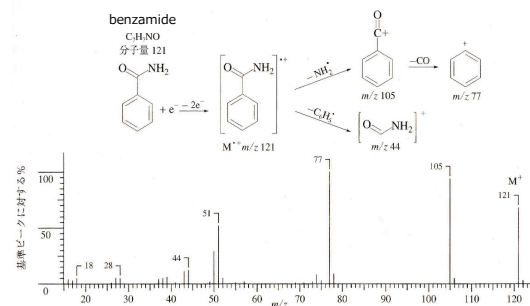## Mass spectra obtained by electron impact ionization method

benzamide
C$_7$H$_7$NO
分子量 121

図 1・1　ベンズアミドの電子衝撃 (EI) 質量スペクトル. スペクトルの上方にいくつかの重要な
フラグメントイオンの生成過程を示す.

The unit of mass is the dalton (Da), defined as 1/12 of the mass of the carbon isotope ^{12}C (12.00000).

50

Principle of Matrix-Assisted Laser Desorption/Ionization

The matrix is excited by the laser beam. The matrix then energizes the sample, causing it to be ionized and vaporized. The external electric field removes the ionized sample from the matrix and it proceeds to the mass separation section.

M : Sample
m : Matrix
C : Cation
A : Anion

Laser
Vacuum
Analyte
Sample Slide
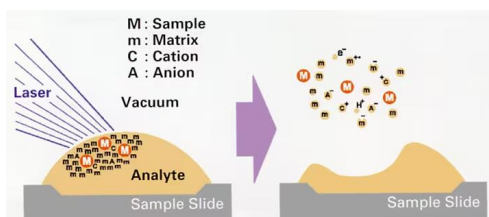
51

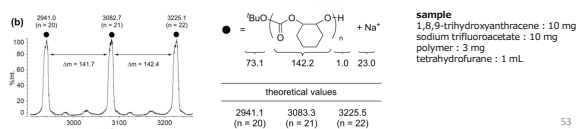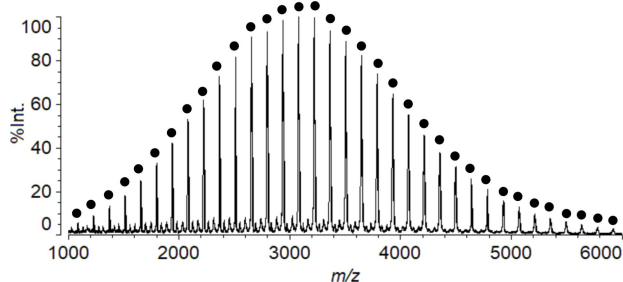
Features of Matrix-Assisted Laser Desorption/Ionization

・Detected Ions : [M+H]$^+$, [M-H]$^-$,etc

・Approximate maximum integer mass that can be measured: 600,000

・The optimal matrix should be used.

・Preparation of samples takes time.

Adaptable to high-mass molecules such as polymers, proteins, and so on.

52

Mass spectrum obtained by MALDI-TOF

sample
1,8,9-trihydroxyanthracene : 10 mg
sodium trifluoroacetate : 10 mg
polymer : 3 mg
tetrahydrofurane : 1 mL

53