Principles and Applications of Vibrational Circular Dichroism (VCD) Spectroscopy

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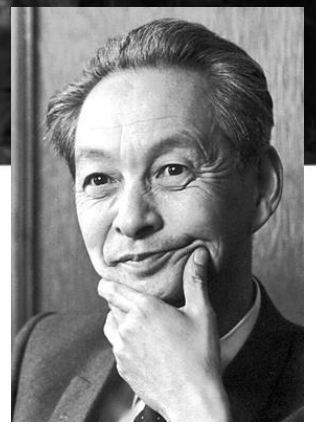
Founding Members

Established 1958 at the Optical Research Institute at Tsukuba University, Tokyo

Founding members include:

- World famous physicist Yoshio Fujioka
- Nobel Prize winner Shinichiro Tomonaga (1965 - Physics for QED with Richard Feynman)

JASCO in the USA, first incorporated in 1972.

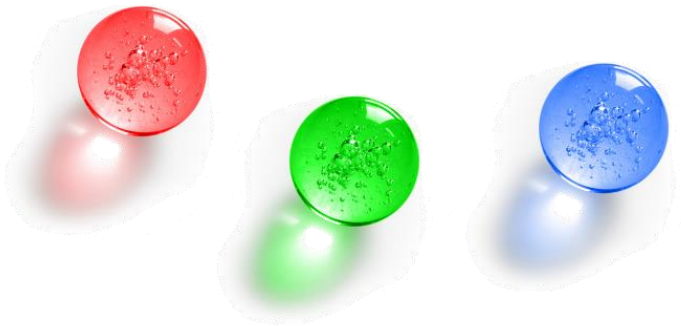


Dr. Tomonaga

JASCO: Our Products

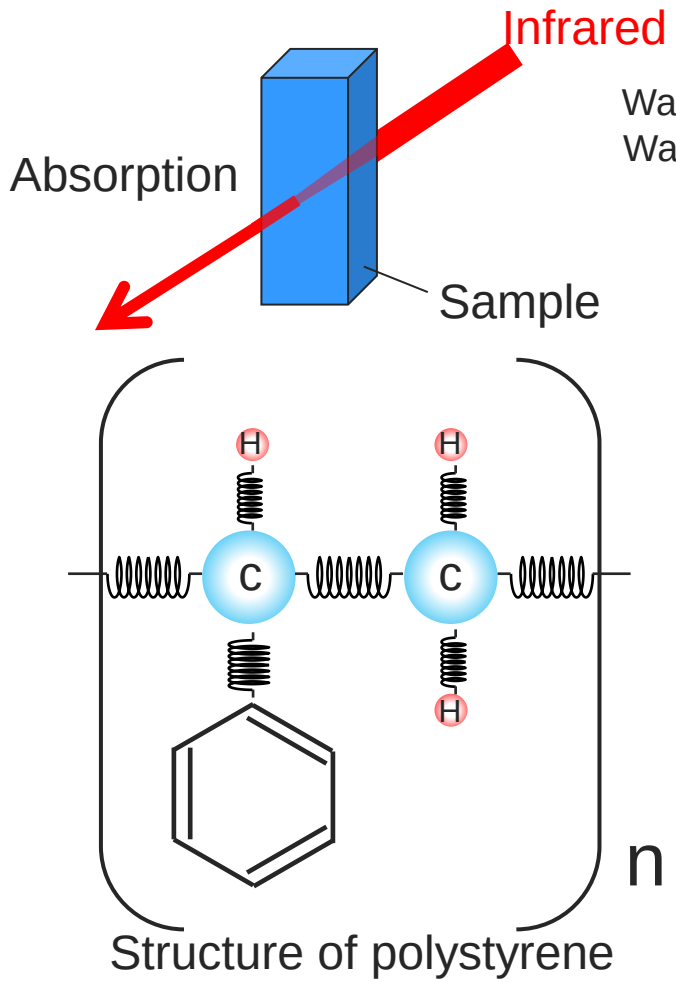
Content

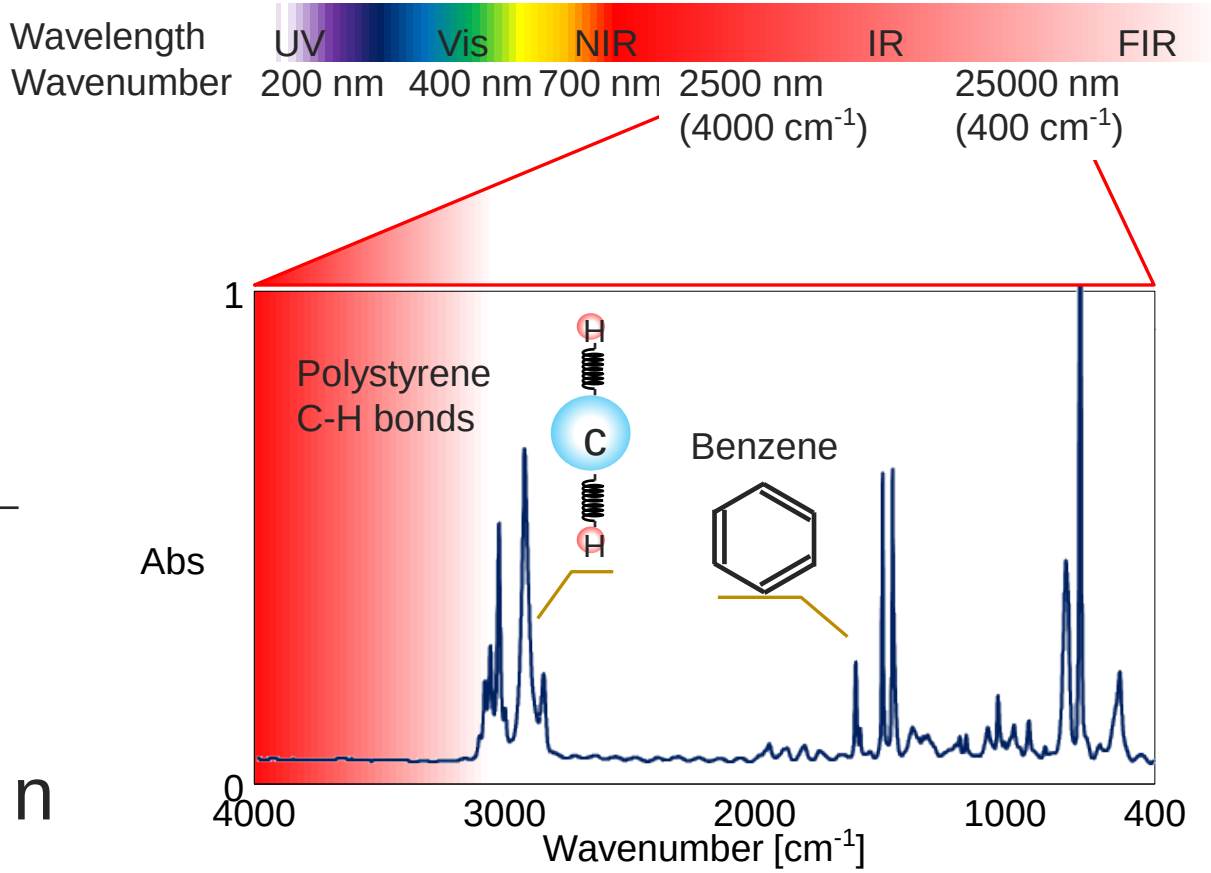
1. Basic principles
2. Instrumentation
3. Measurement procedures
4. Applications



Infrared Spectroscopy

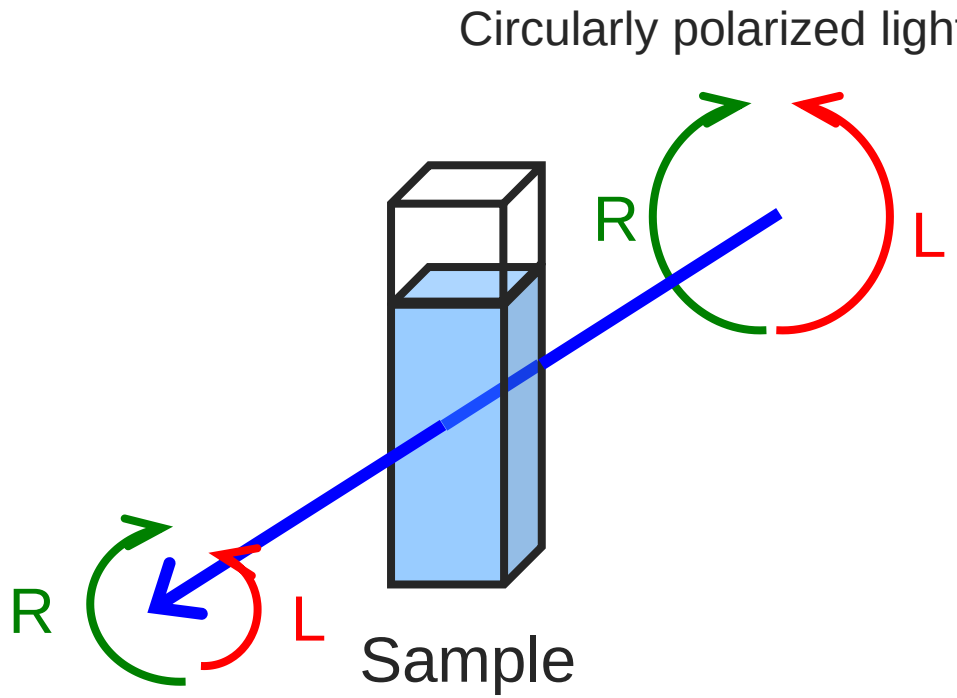
Irradiating a sample with infrared light and measuring its absorption spectrum





Since absorption is specific to each functional group, infrared spectroscopy is used for qualitative analysis and molecular structure determination.

Circular Dichroism Spectroscopy



Definition

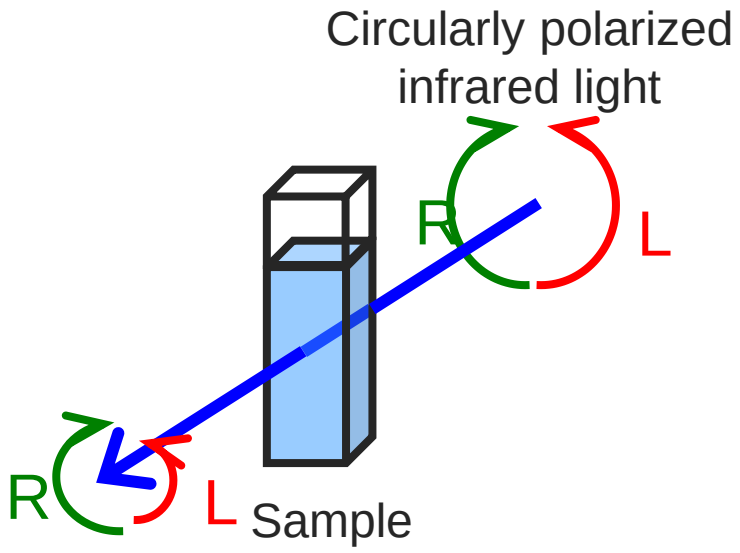
$$DAbs = A_L - A_R$$

What is VCD?

VCD : Vibrational Circular Dichroism

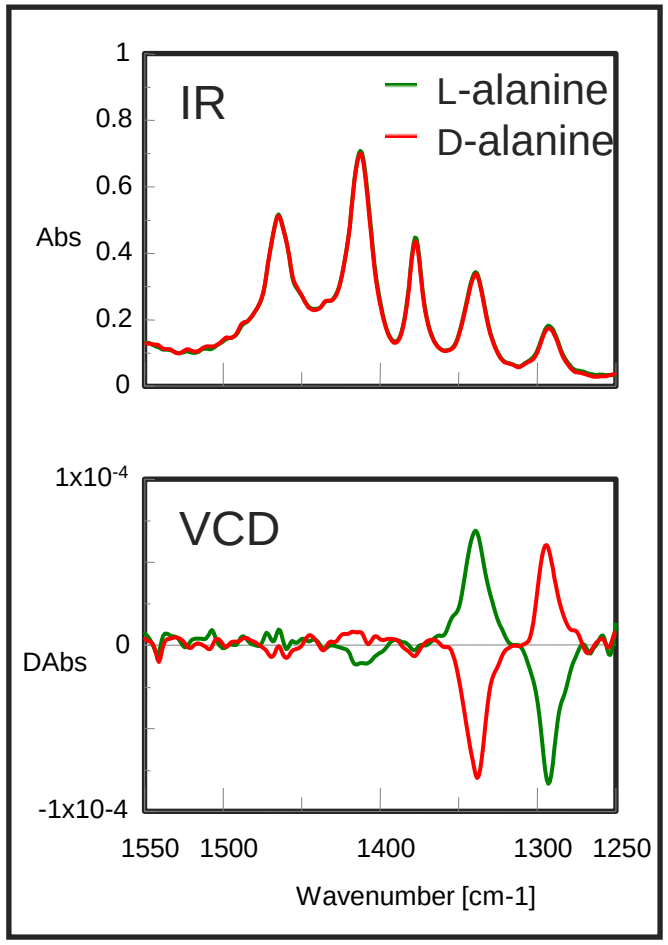
Vibrational: absorption in the infrared region due to molecular vibrations
Circular dichroism: degree of absorption of left- and right-handed circularly polarized light

VCD: difference in absorption of left- and right-handed circularly polarized infrared light



ECD: Electronic Circular Dichroism circular dichroism in ultraviolet/visible region

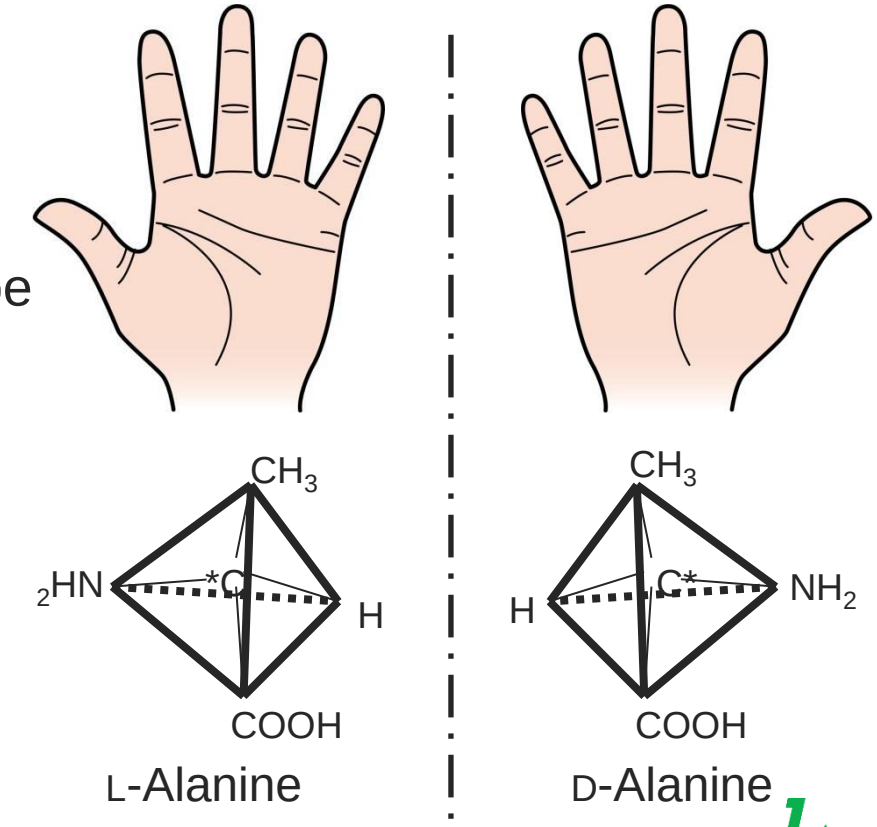
VCD Spectrum D and L Alanine



Substances Exhibiting CD

Substances that exhibit CD are optically active compounds

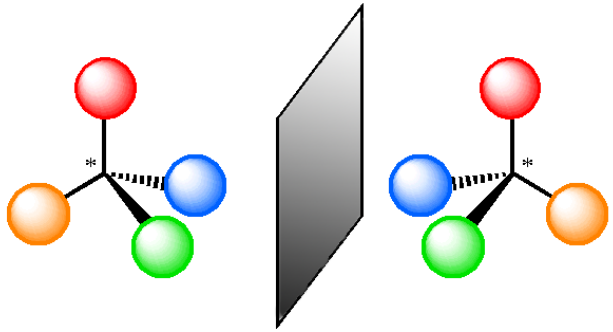
- Compounds with a combination of enantiomers that cannot be superimposed on their mirror image
- Compounds consisting of just one of these enantiomers



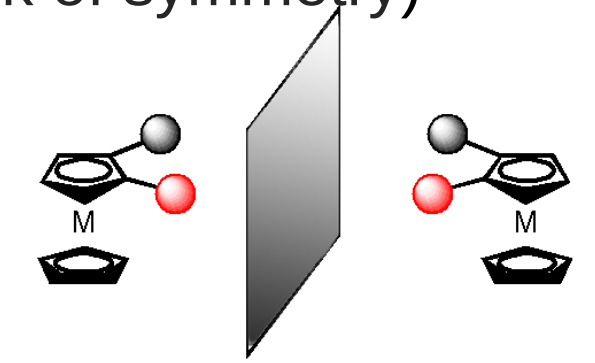
Substances Exhibiting CD

Types of chirality (chiral: lack of symmetry)

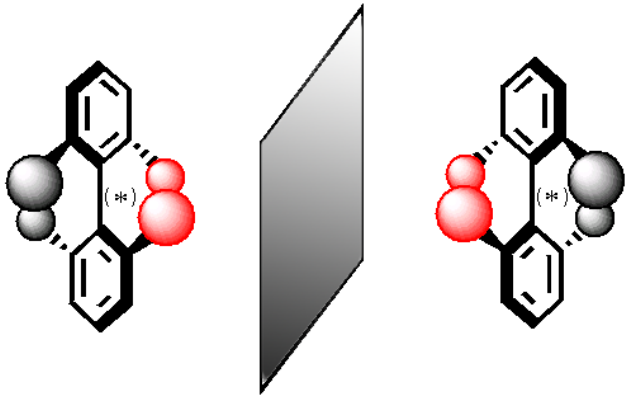
*Asymmetric carbon atom



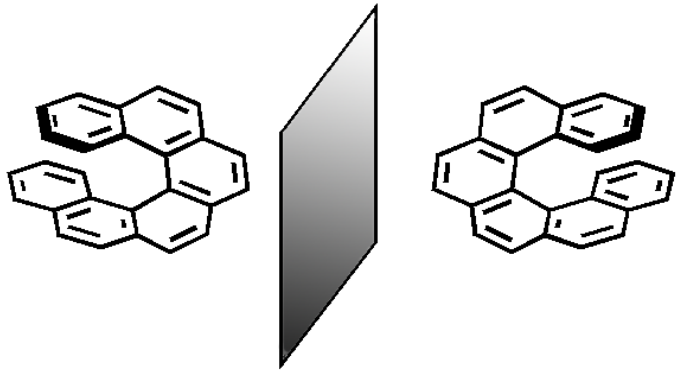
Point chirality



Planar chirality



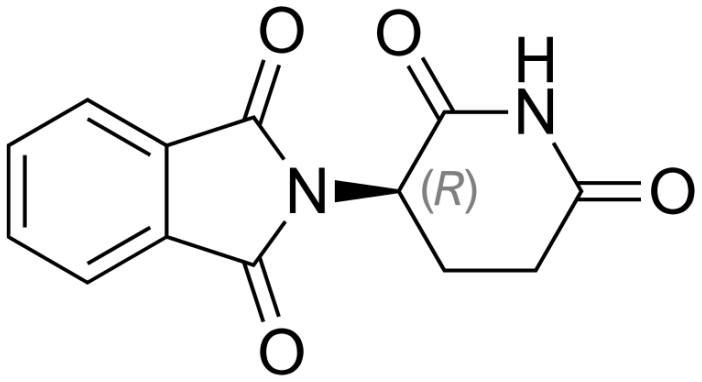
Axial chirality



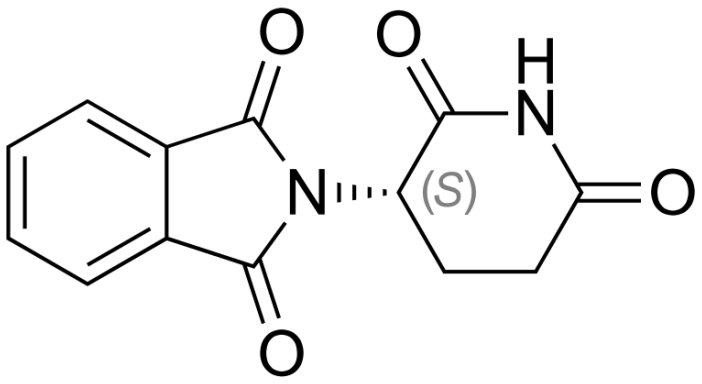
Helical chirality

Importance of Absolute Configuration and Stereochemistry

Thalidomide



Effective against illness



Causes birth defects

What is Learned from VCD Spectroscopy?

1. All organic compounds absorb infrared light and can be analyzed without the use of a chromophore

2. Chiral compounds can be identified

3. The absolute configuration of a sample can be determined by comparing its spectrum to that calculated using molecular orbital methods

Determining Absolute Molecular Configuration

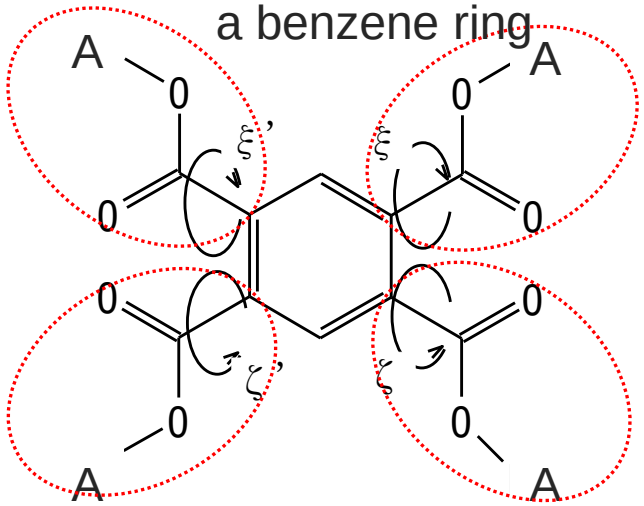
VCD can determine the absolute configuration of a molecule.

Procedure

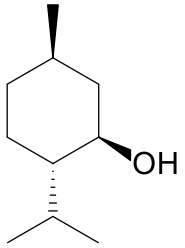
1. Calculate the optimized molecular geometry
2. Calculate the VCD spectrum for the optimized molecular structure
3. Measure the VCD spectrum of the sample
4. Compare the calculated and measured spectra to determine the absolute sample configuration

Structural Optimization Calculation

Molecule in which four ester groups are bonded to a benzene ring



A ⟺ Menthol

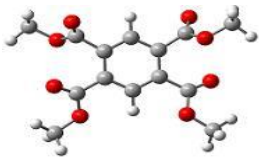


By allowing these ester groups to rotate freely, various conformations occur

The results of the structural optimization calculation

B3LYP/6-31G*//B3LYP/6-31G*

Form 1



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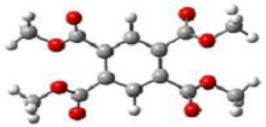
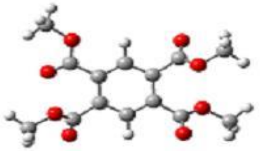
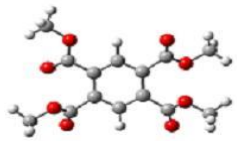


Form 3



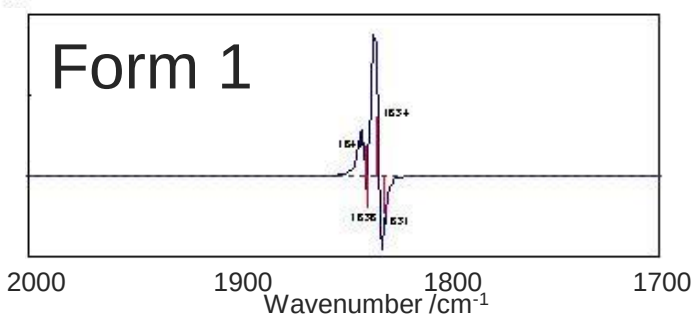
Form 4

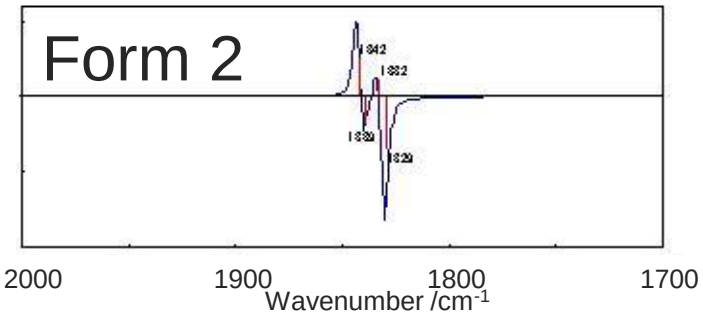


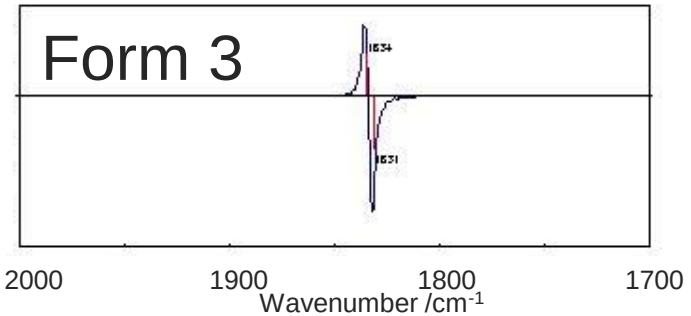
Form 5

Chiral symmetry across the benzene ring (planar chirality)

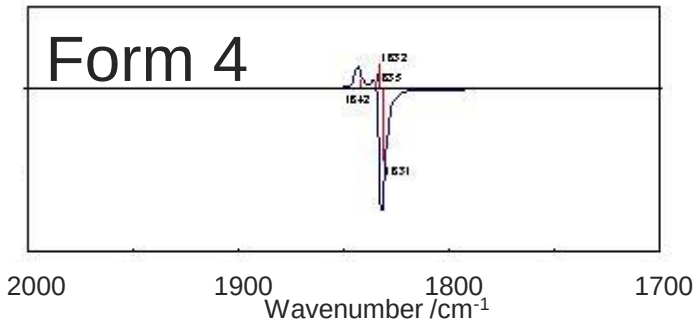
Calculated VCD Spectra for 5 Stable Conformations

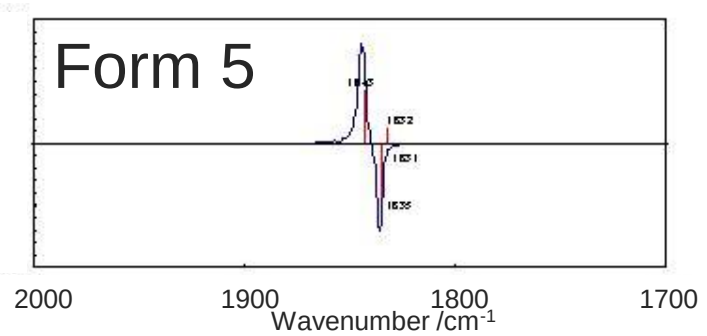






Calculated based on carbonyl group

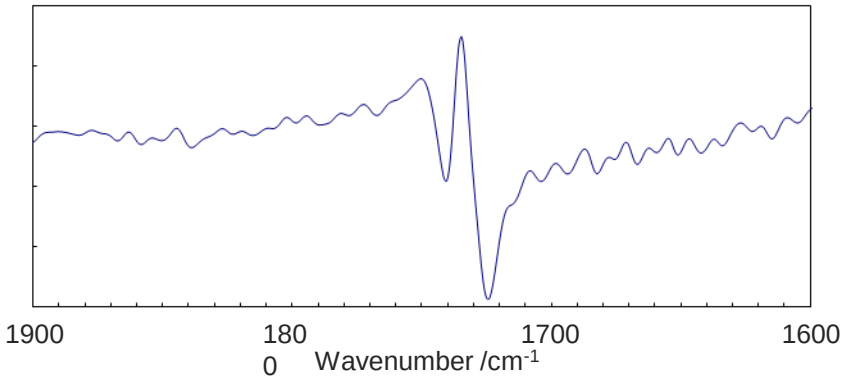




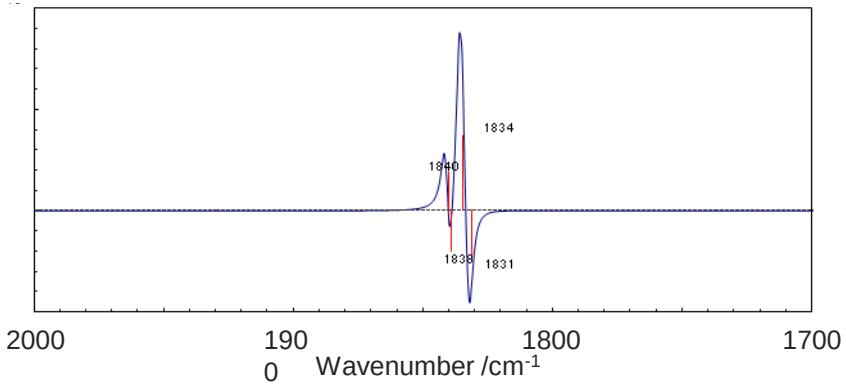
The spectrum varies depending on the molecular structure

Comparison of Calculated and Measured Spectra

Measured spectrum



Calculated spectrum



Form 1

- Measured spectrum is similar to calculated spectrum of Form 1
- Form 1 is the most likely molecular conformation in this sample

Considerations of VCD Measurement

1. A high sample concentration is needed, implying a large amount of sample
   Concentration: approximately 10 to 100 mg/ml

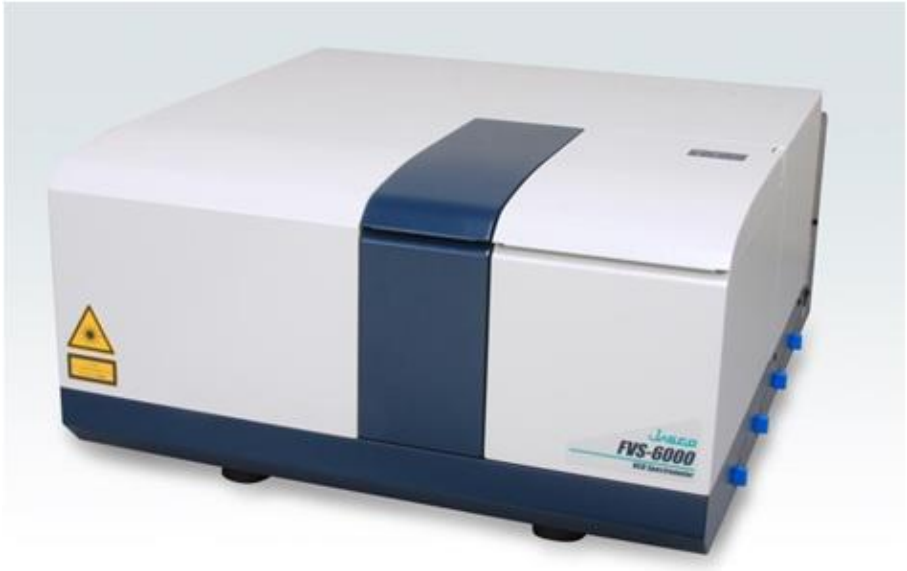
2. VCD signals are weak, so a long accumulation time is required
   VCD: $DA/A = 10^{-3}$ to 10^{-5}
   ECD: $DA/A = 10^{-1}$ to 10^{-3}

Highly sensitive and stable instrument is required

JASCO VCD System

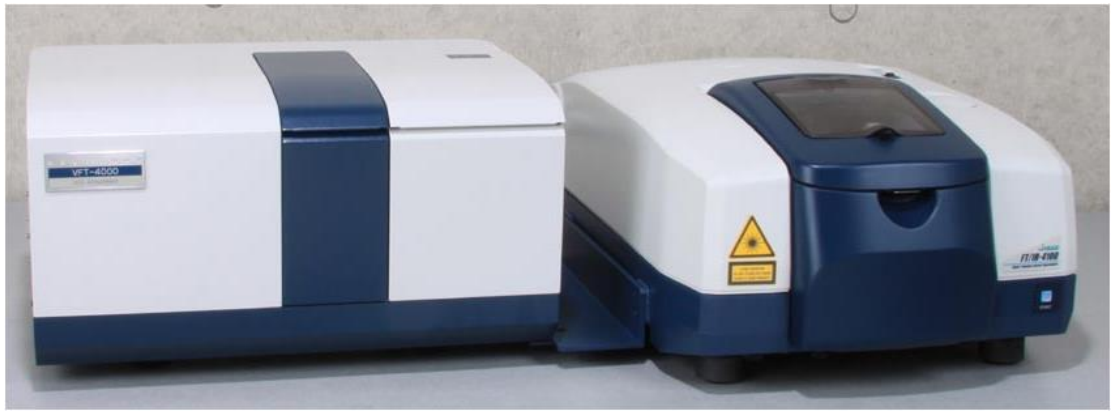
Dedicated spectrometer for VCD measurements

- Highly sensitive system for detecting weak VCD signals



FVS-6000

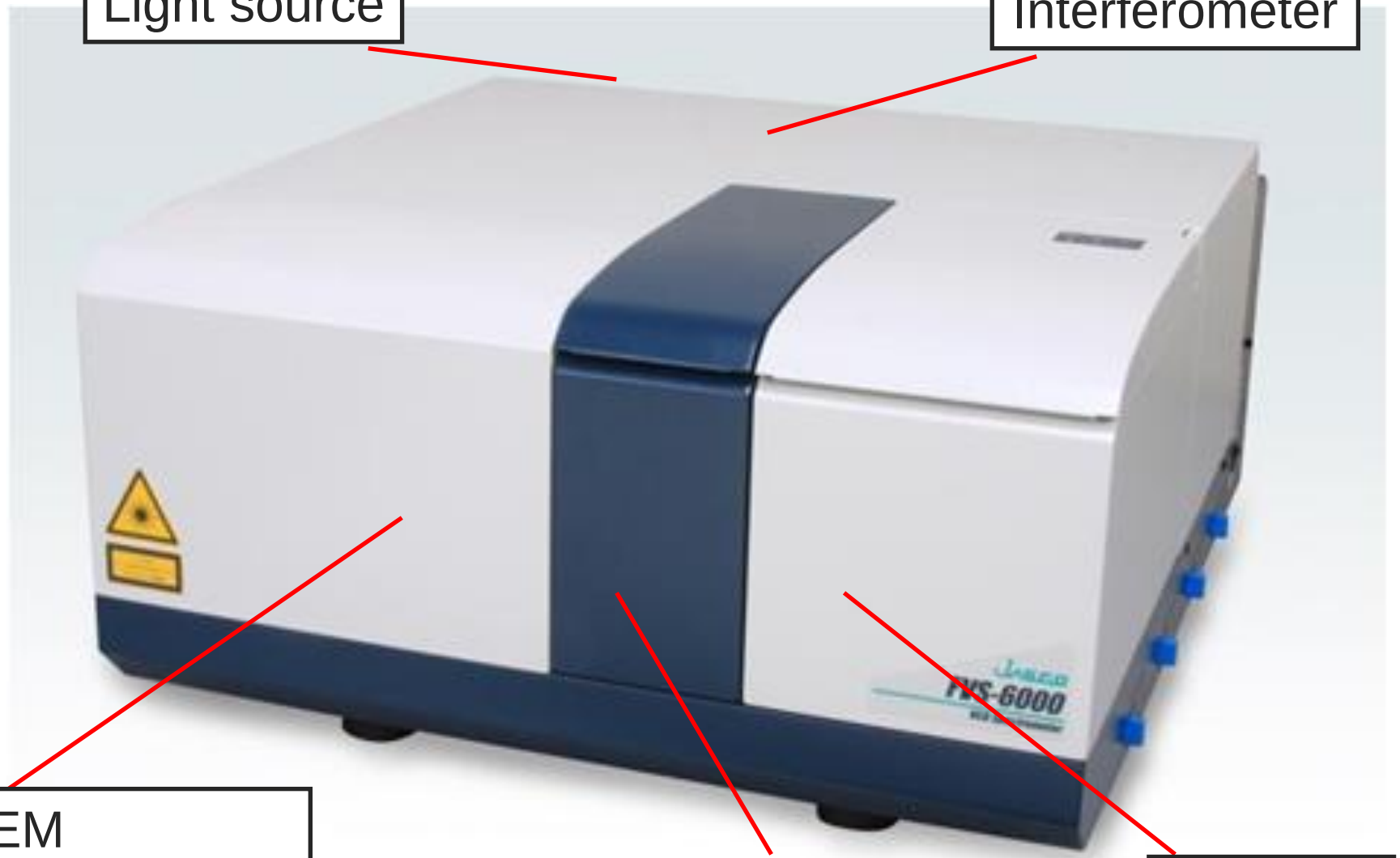
Accessories



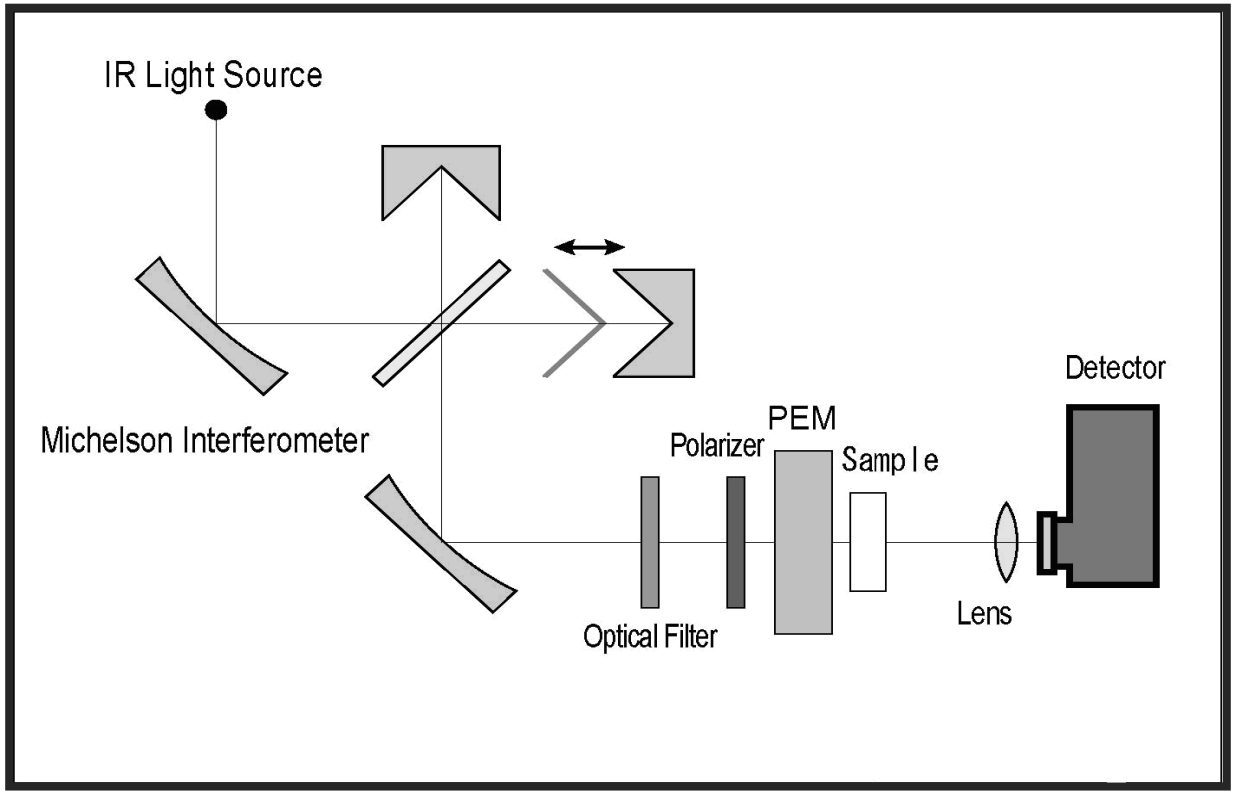
VFT-4000 FT/IR-4700

- FTIR accessory
- Highly extendable (ATR, etc.)

FVS-6000 System



Block Diagram of the VCD system



1. Infrared light is transformed to the Fourier frequency domain using a Michelson interferometer
2. Only light in the measurement range is transmitted through the optical filter
3. Linearly polarized light is produced using a polarizer
4. Left- and right-handed circularly polarized light is generated by the PEM operating at 50 kHz.
5. Infrared light is detected using a high-speed / high sensitivity detector

Approaches for Measuring Weak VCD Signals using FVS-6000

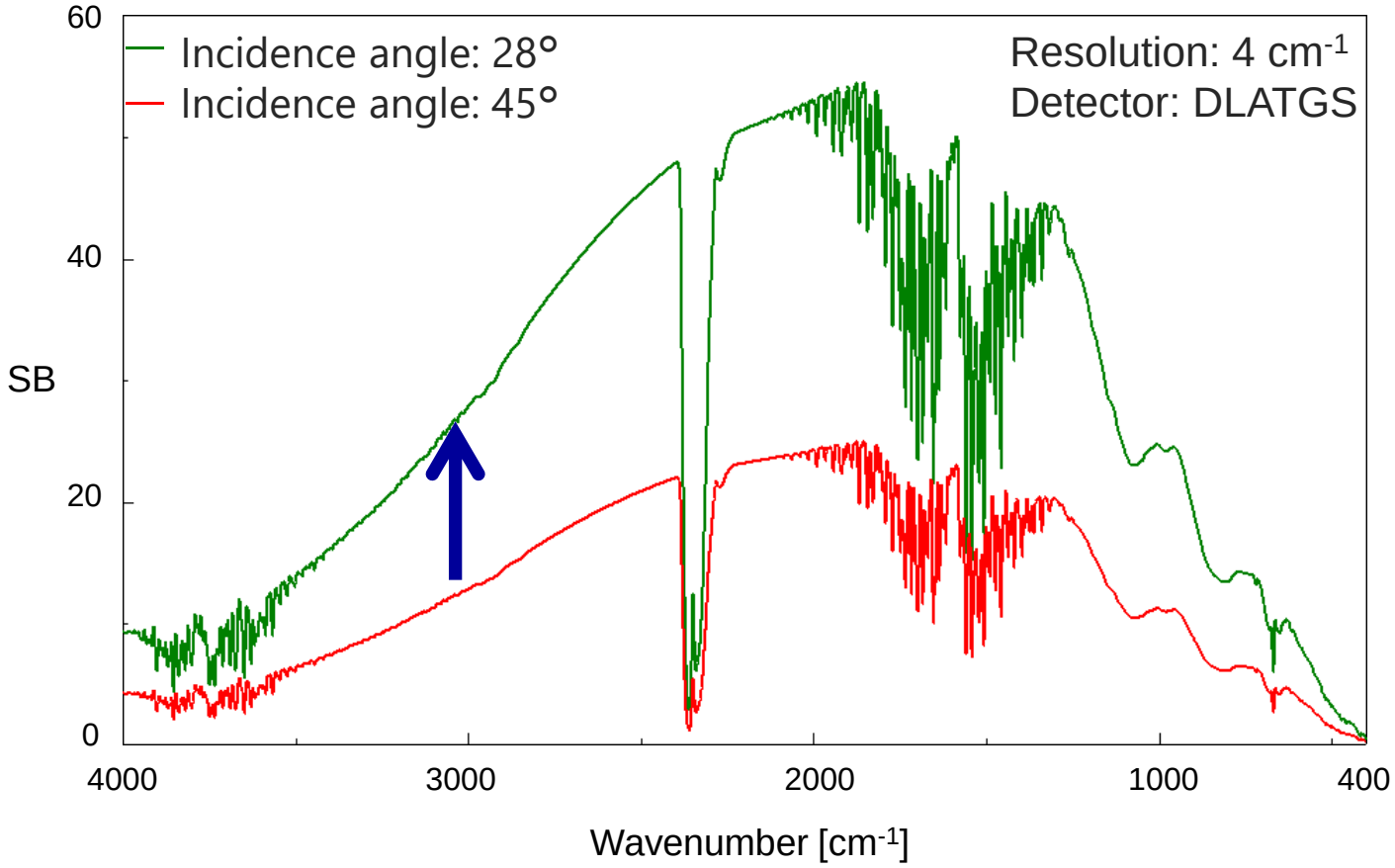
1. Improved sensitivity using high-throughput interferometer

2. Combination of highly sensitive detector and optical filter

3. Symmetry and stability

Effect of Interferometer Incidence Angle on IR Intensity

Dependence of IR light intensity on incidence angle of interferometer



Increased amount of light by changing incidence angle of interferometer leads to 2.2 times sensitivity improvement

Advantages of FVS-6000 for Weak VCD Signals

1. Improving the sensitivity using high-throughput interferometer
2. Combination of highly sensitive detector and optical filter
3. Symmetry and stability

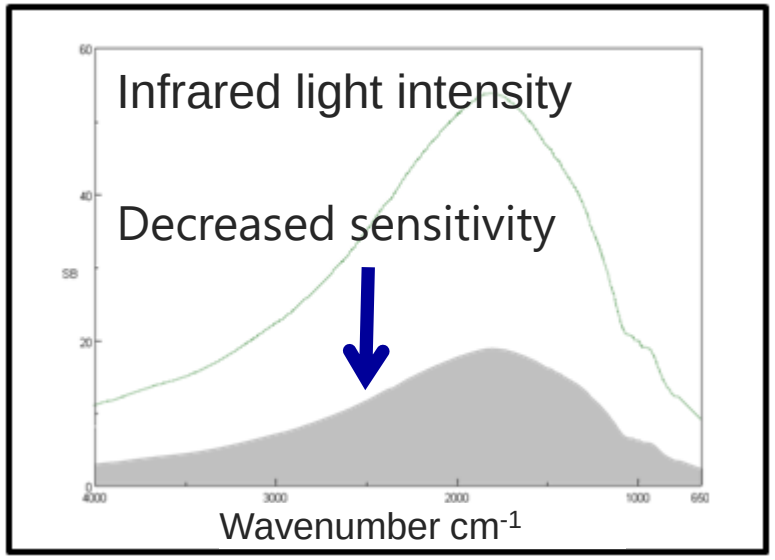
MCT Detector

- Liquid N_2 cooling is needed for the MCT detector
- Compared with the DLATGS detector (operating at room temperature) generally used in FTIR spectroscopy, the sensitivity of the MCT detector is greater than an order of magnitude higher
- In the solution method (transmission method) used for VCD measurements, the light is too intense to be directly transmitted to the detector without saturating it
- A metal mesh or an optical filter is used so that the detector is not saturated

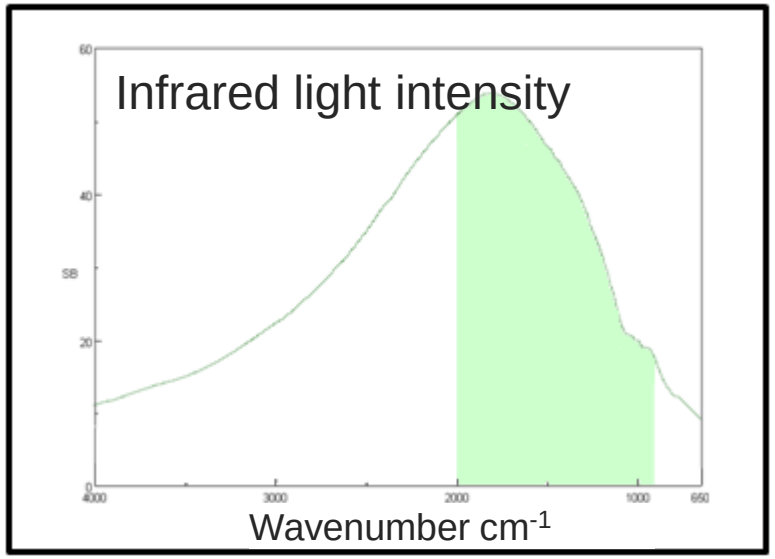
Metal Mesh and Optical Filter

- Metal mesh: decreases sensitivity because light at all wavenumbers is attenuated
- Optical filter: does not attenuate light but passes only light in a certain wavenumber range, allowing highly sensitive measurements

Metal mesh

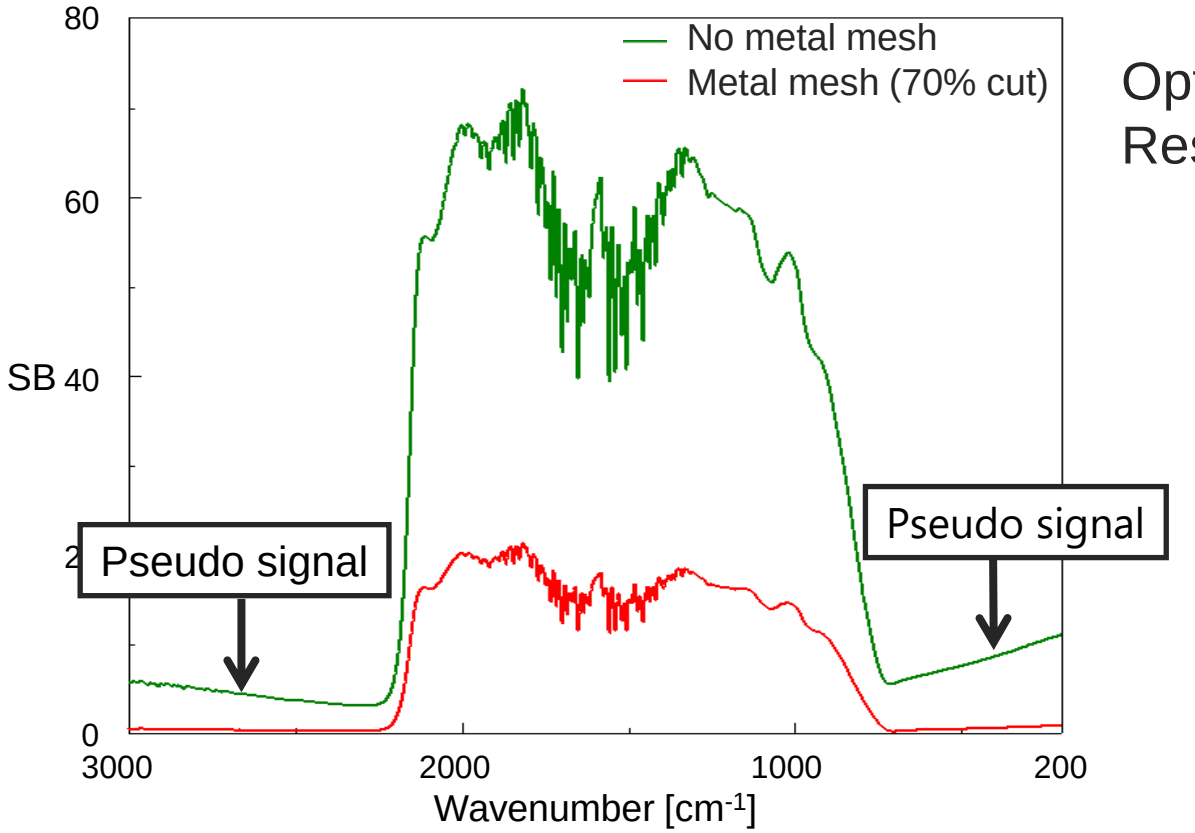


Optical filter



Photoconductive MCT (PC-MCT) Detector

Single beam spectrum using photoconductive MCT detector

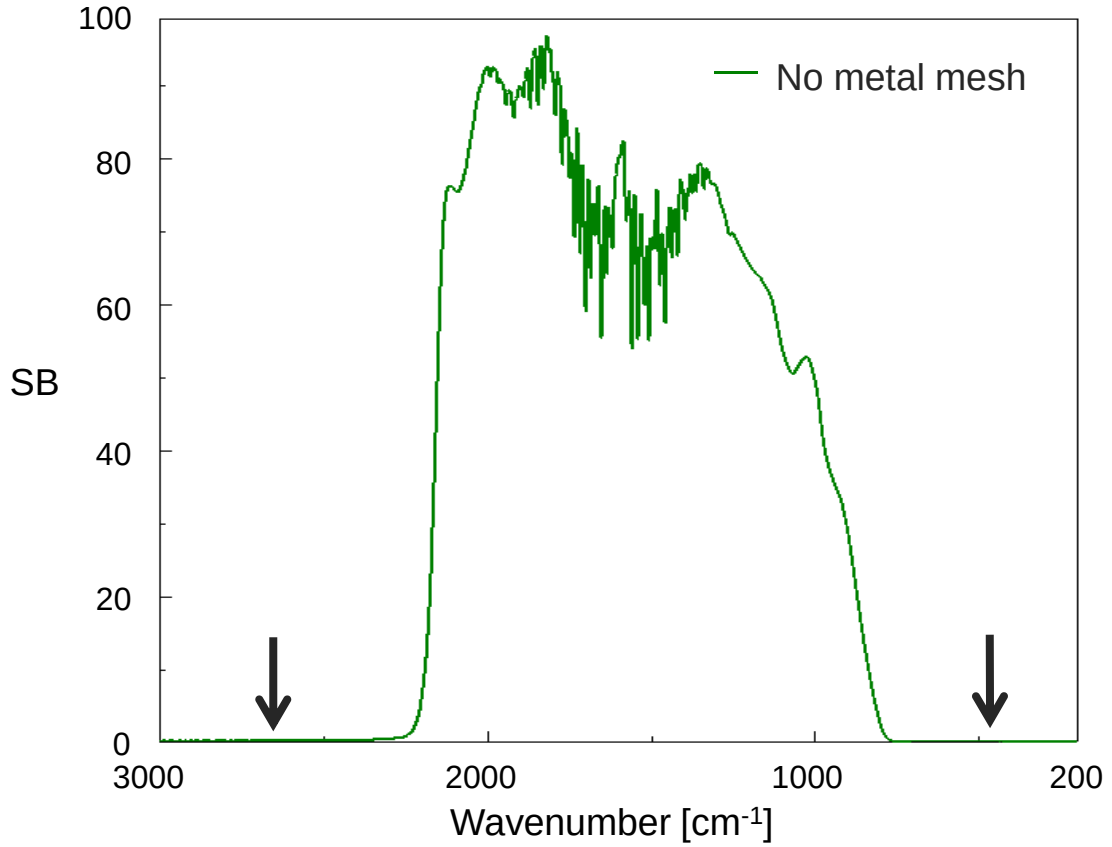


Optical filter: 5 μm LPF
Resolution: 4 cm^{-1}

- If no metal mesh is used, the detector is saturated, and false signals appear
- The sensitivity decreases because the light is cut by about 70% with a metal mesh

Photovoltaic MCT (PV-MCT)

Single beam spectrum using photovoltaic MCT detector



Optical filter: 5 μm LPF
Resolution: 4 cm^{-1}

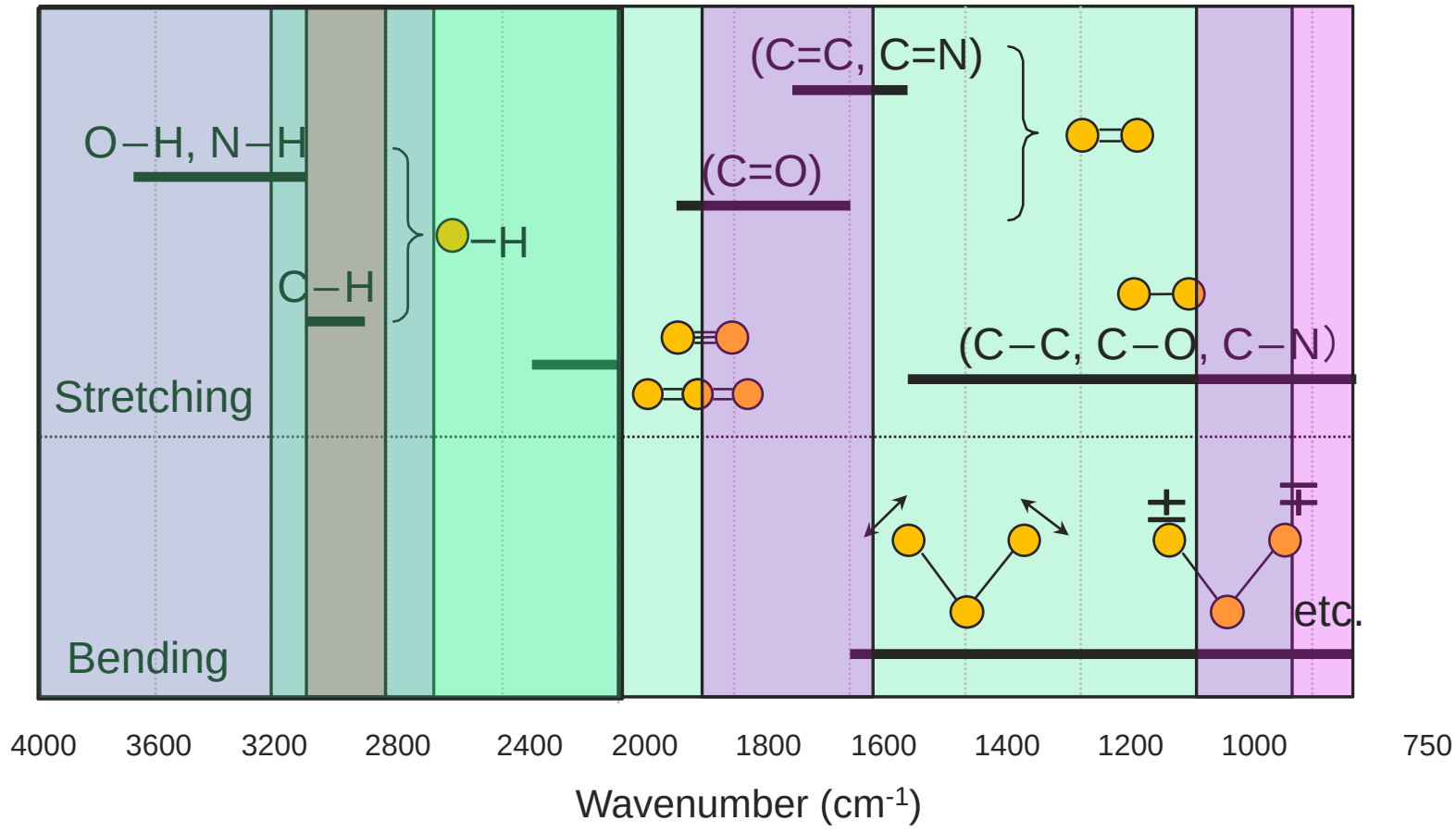
- With a PV-MCT, the detector is not saturated even if no metal mesh is used.
- This provides a measurement sensitivity about 3 to 4 times higher than that for a PC-MCT.

Absorption Wavenumbers for Filters and Functional Groups

Optical filter lineup

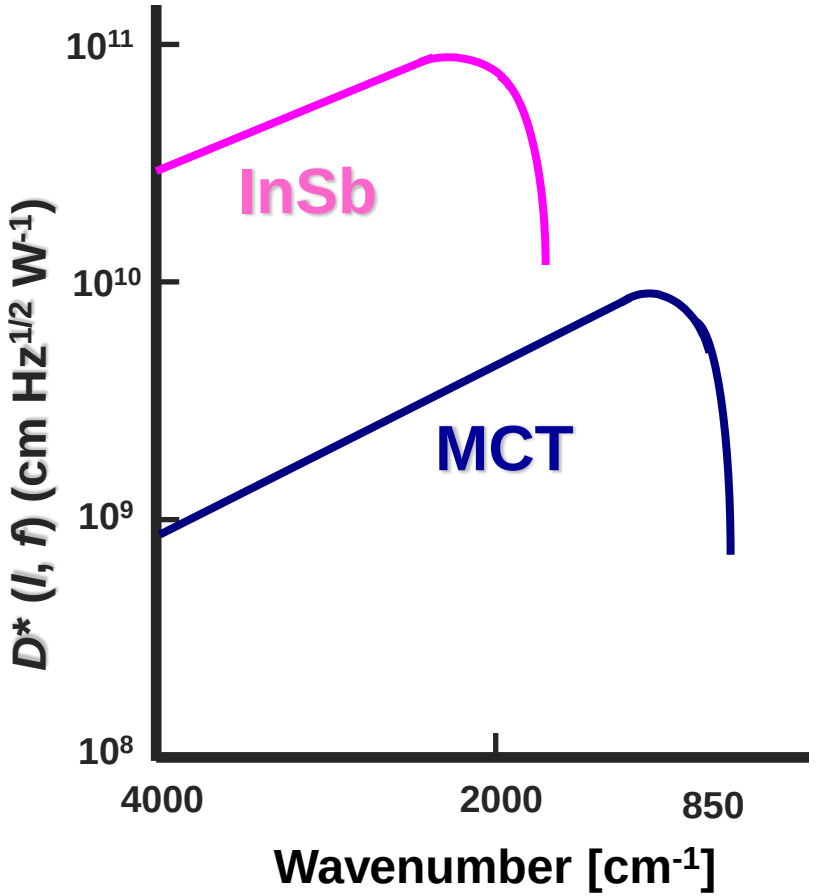
Standard: 2000 to 850, 3200 to 2000 cm^{-1}

Optional: 4000 to 2650, 1000 to 750, 1850 to 1550 cm^{-1}, 3050 to 2800 cm^{-1}



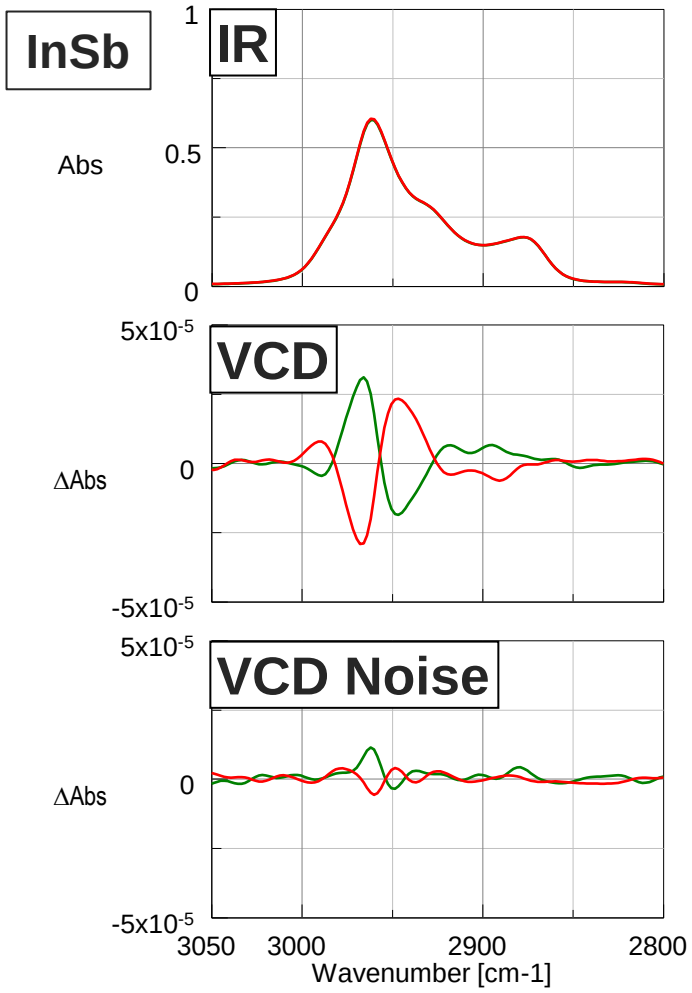
Measurements at High Wavenumbers

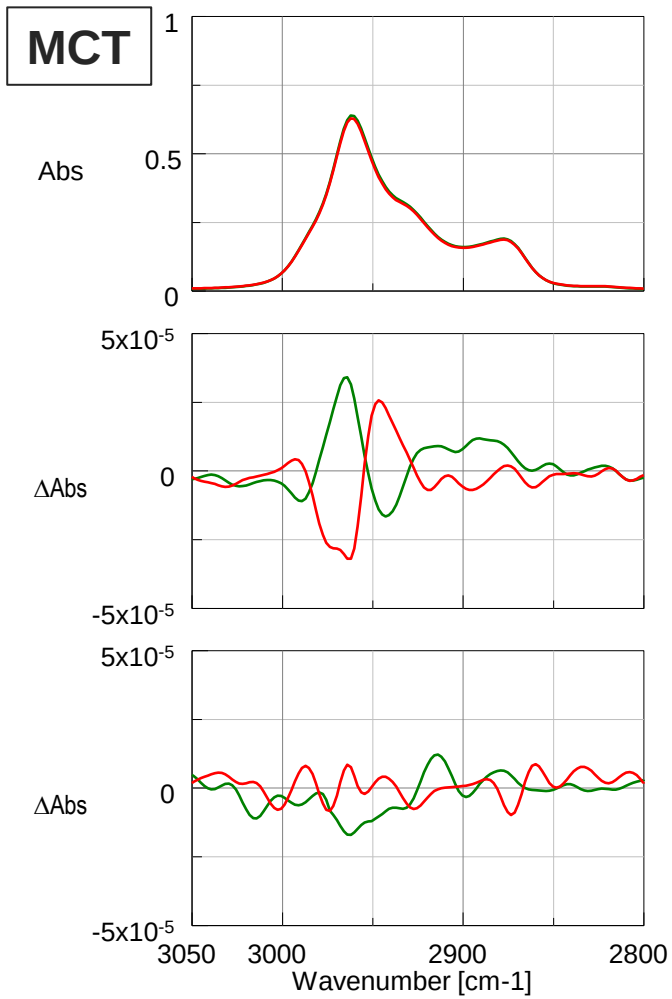
Sensitivity characteristics of detectors

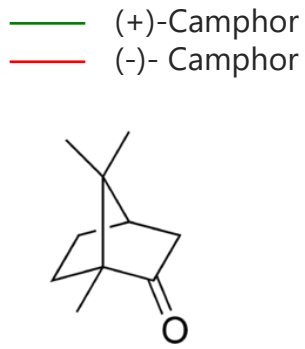


- Using a MCT detector, the sensitivity in the high-wavenumber region decreases
- An InSb detector is effective at 2000 cm^{-1} or higher.

Comparison of InSb and MCT Detectors





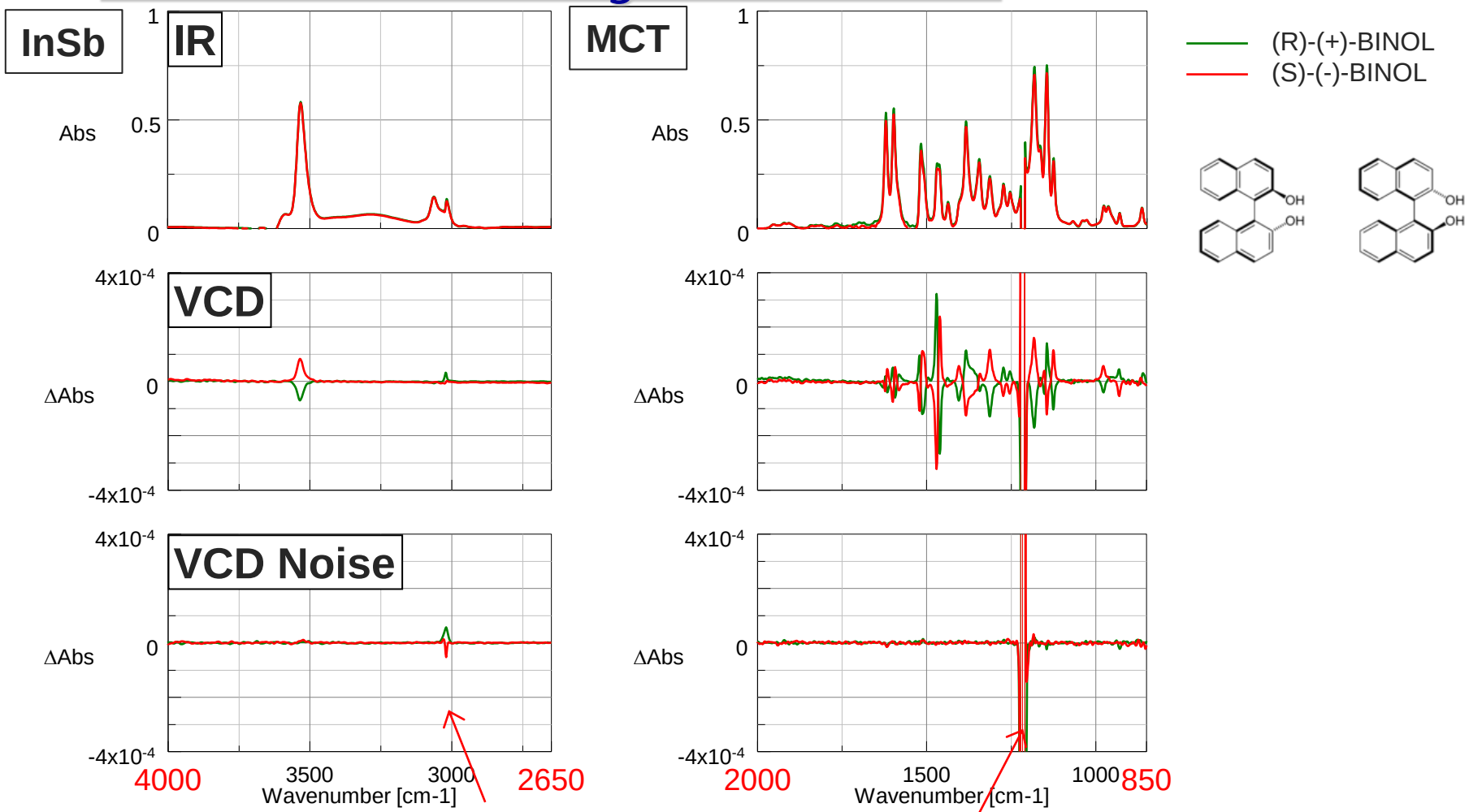


No. of accumulations: 500

An InSb detector is a better choice at high wavenumbers

Choice of Detector

Best to use an MCT detector at 2000 cm^{-1} or lower and an InSb detector at high wavenumbers



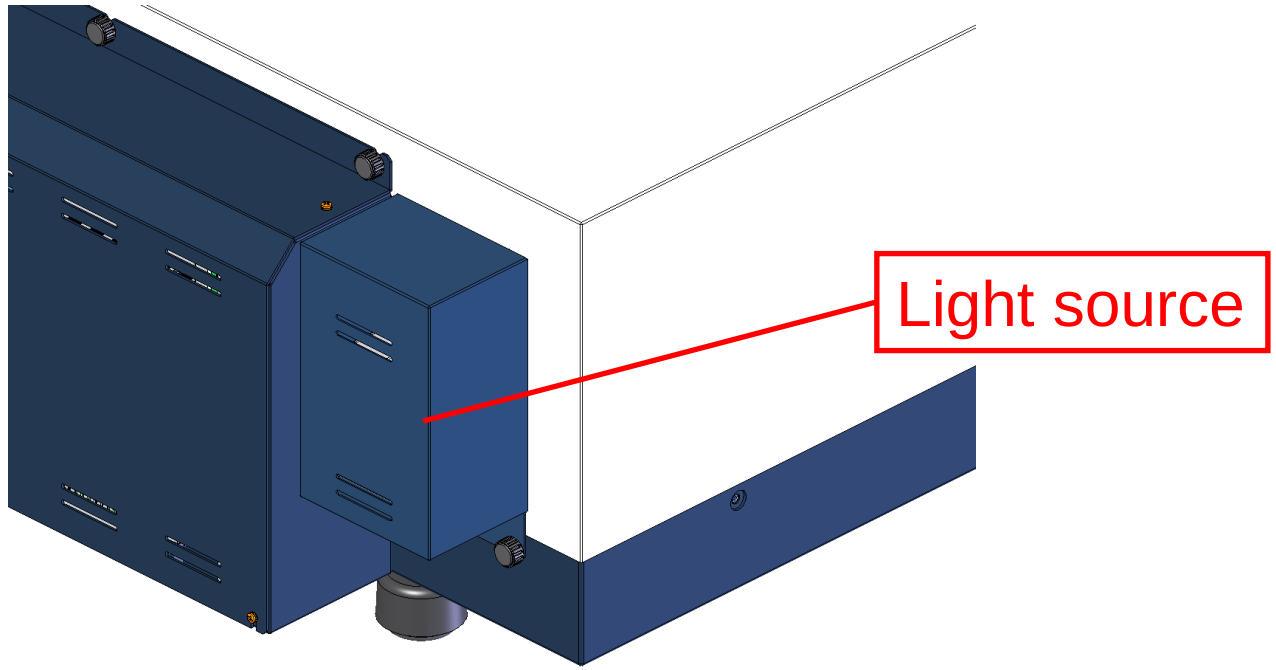
*The noise is excessive due to solvent absorption.

Advantages of FVS-6000 for Weak VCD Signals

1. Improved sensitivity using high-throughput interferometer
2. Combination of highly sensitive detector and optical filter
3. Symmetry and stability

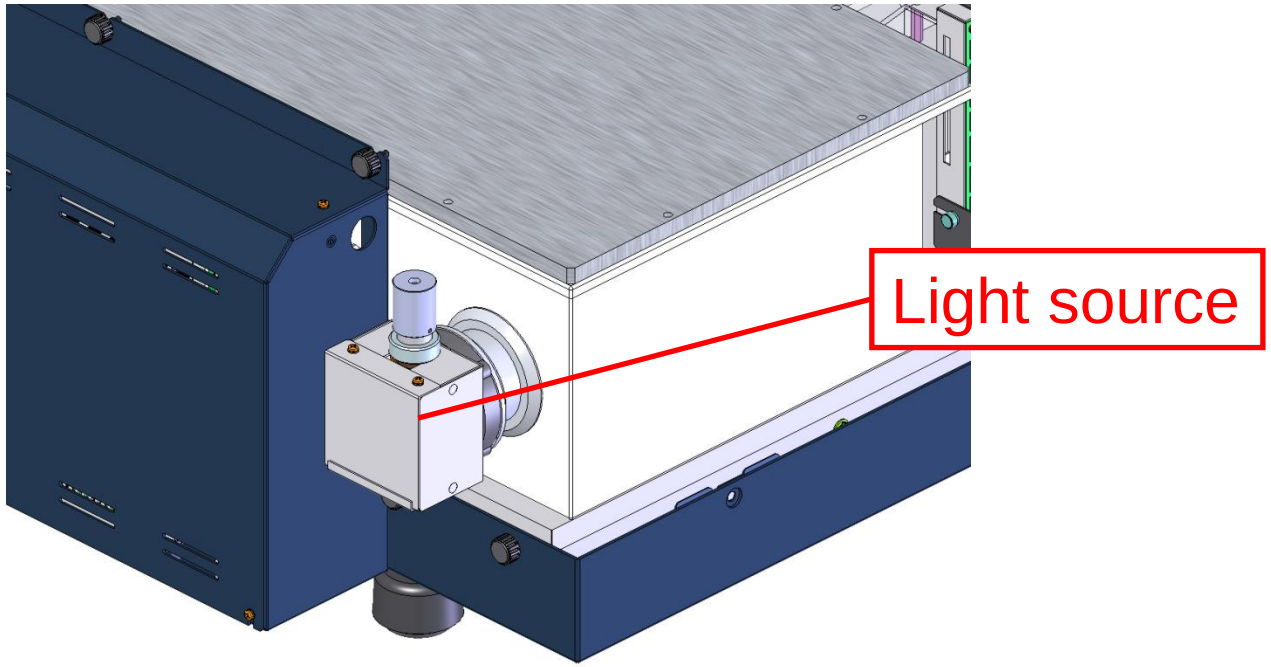
Instrument Stability

- Instrumental stability is important because VCD signals are weak
- The baseline variations and the sign of peaks becomes unclear due to thermal variations in the light source



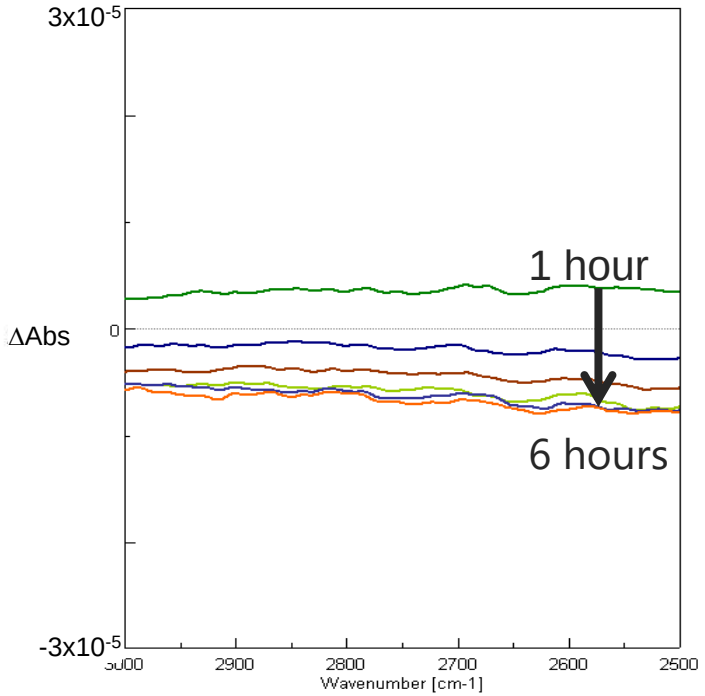
Instrument Stability

By mounting the light source independently from the optical system, the influence of thermal variations on the baseline is minimized.

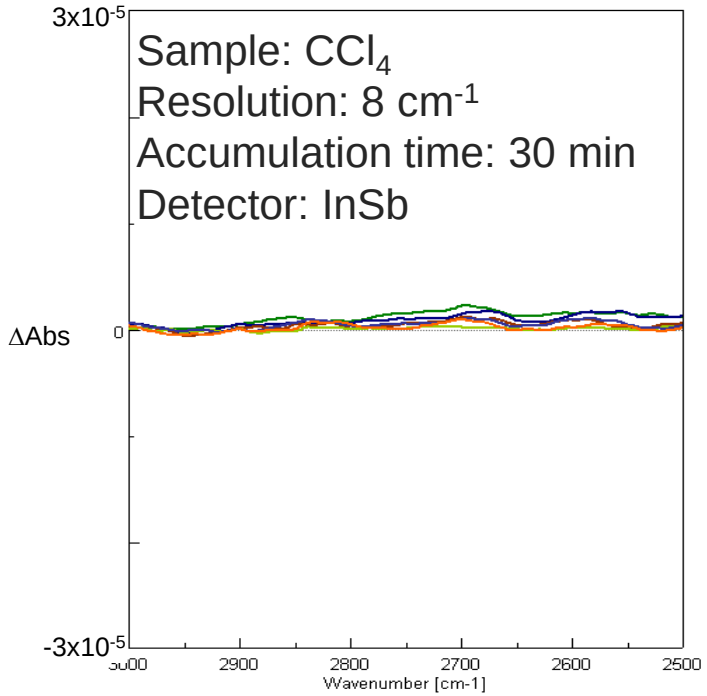


Temperature Stabilization of Optical System

Baseline stability (carbon tetrachloride)



Thermostat OFF



Thermostat ON

Measurements were performed every hour for 6 hours after turning on the instrument

Temperature stabilization of optical elements also stabilizes the baseline

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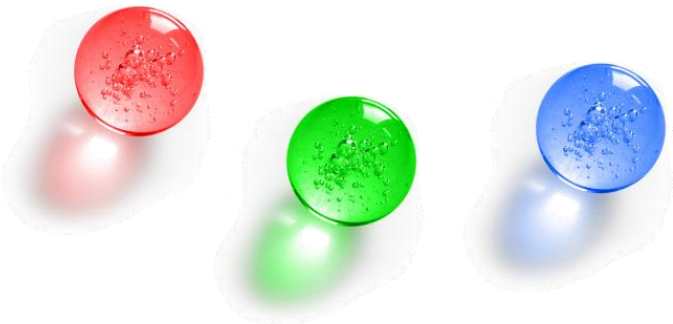
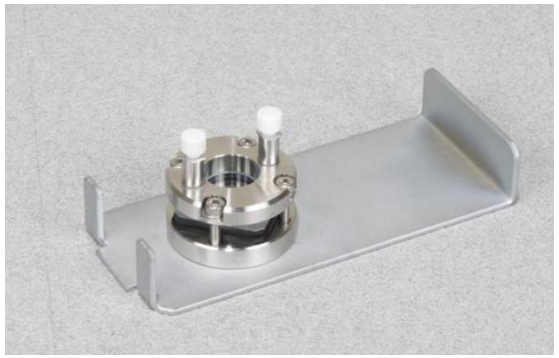


Sample Preparation

Liquid sample cell



Window	Range	Remarks
BaF_2	~ 800 cm^{-1}	Slightly soluble in water
CaF_2	~ 1100 cm^{-1}	Insoluble in water

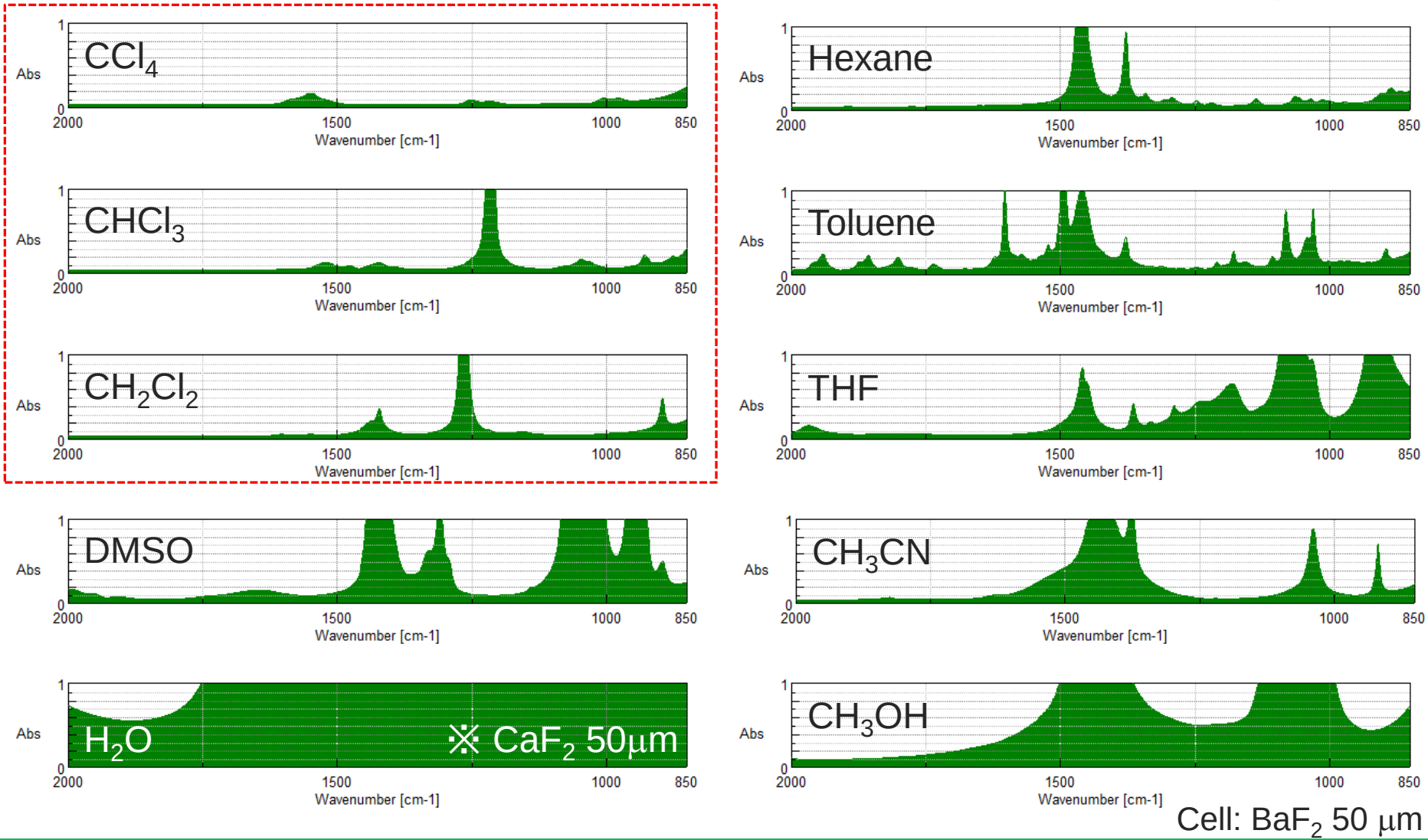
Aqueous solution samples ➡ CaF_2
Non-aqueous solution samples ➡ BaF_2

- Sample amount: 1 to 10 mg
- Sample volume: ~100 µl
- Sample concentration: 10 to 100 mg/ml

Solubility should be checked in advance due to the high sample concentration

Choice of Solvent (IR Spectrum: 2000 to 850 cm^{-1})

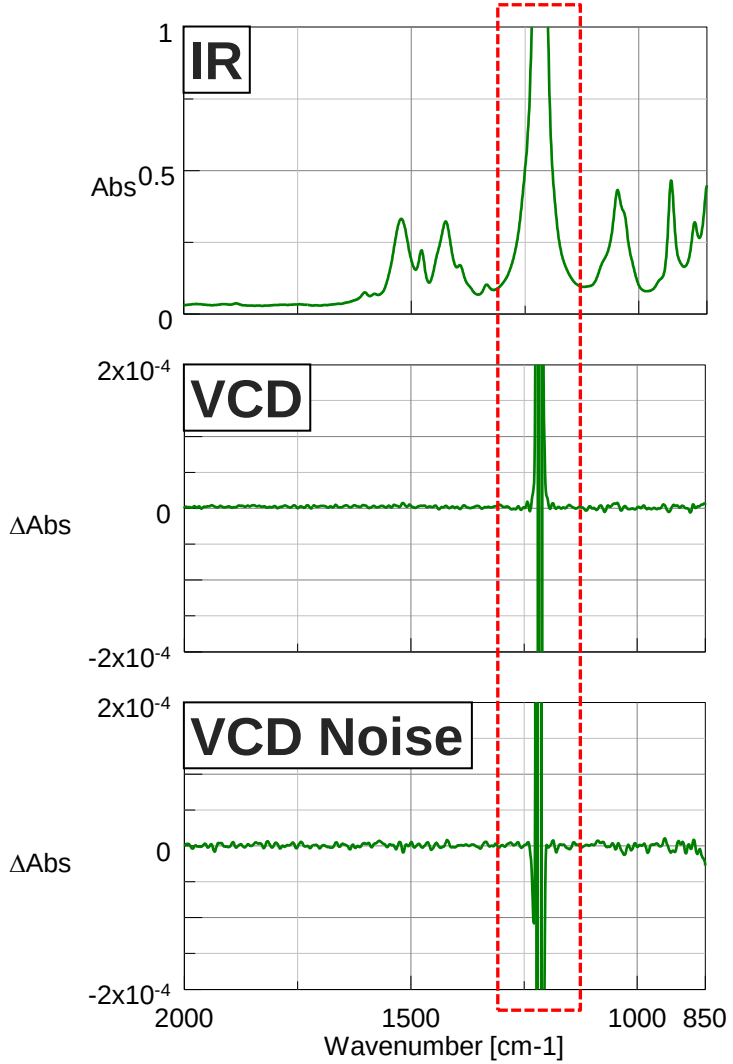
Better results are obtained with a solvent with less IR absorption



Cell: BaF_2 50 μm

VCD Spectra of Solvent

Chloroform: $CHCl_3$



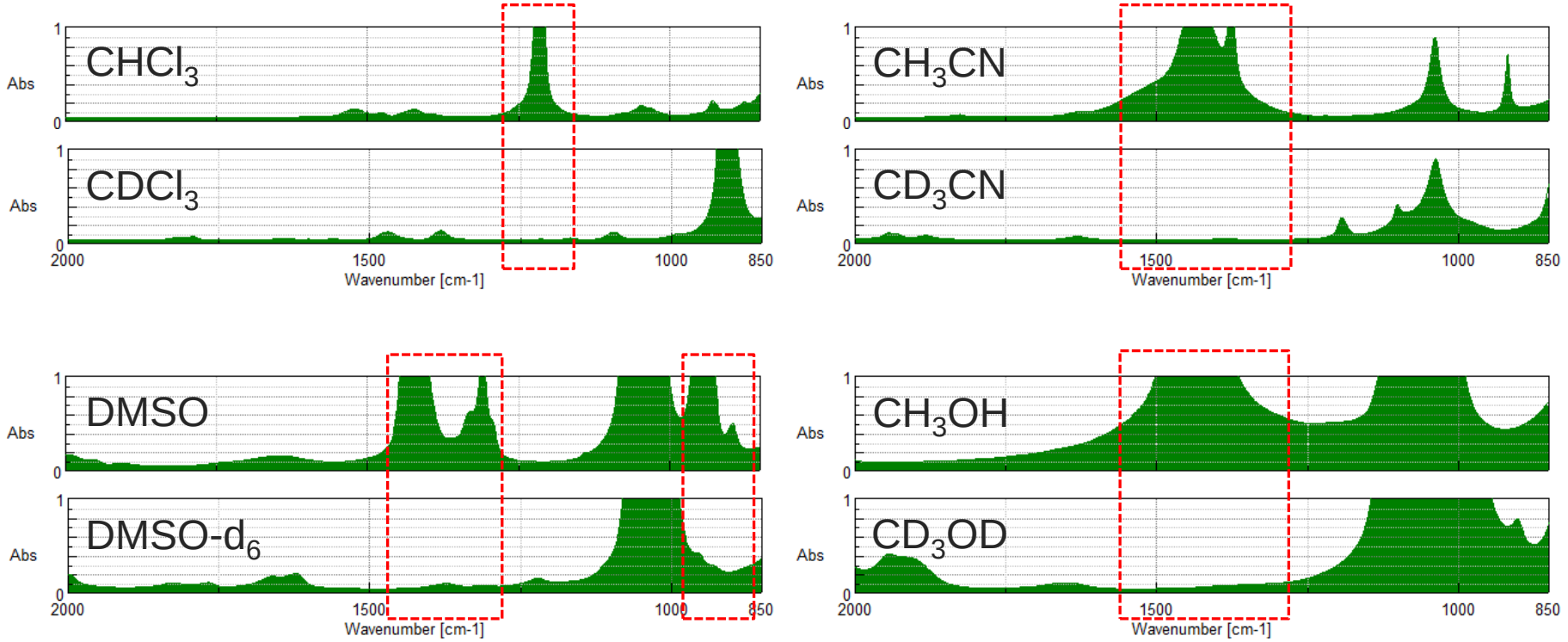
In strong IR absorption range, impossible to measure a VCD spectrum of a sample

Solvents Deuterium Substitution (2000 to 850 cm^{-1})

Solutions for solvents with strong absorption

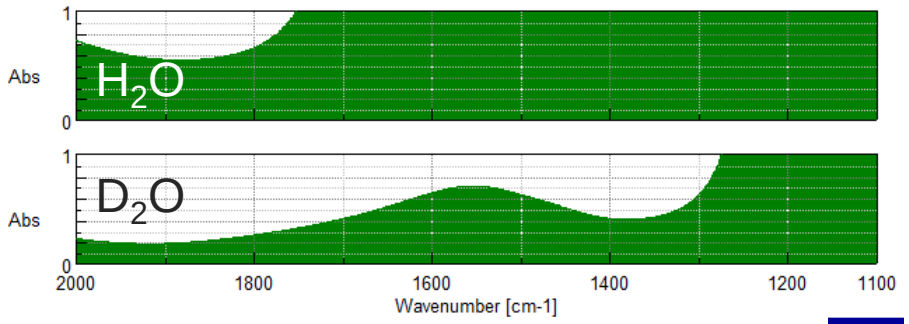
➡ 1. Use deuterated solvents to reduce the effects of solvent absorption

Cell: BaF_2 50 μm



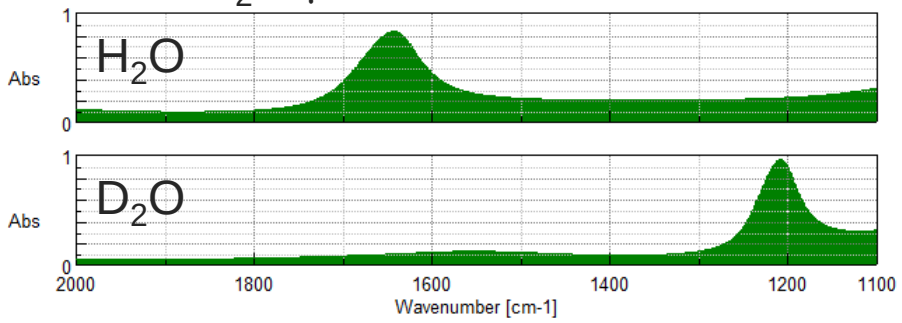
Optical Path Length (2000 to 850 cm^{-1})

Cell: CaF_2 50 μm



In the case of water, when heavy water is used, the absorption decreases but remains strong

Cell: CaF_2 4 μm

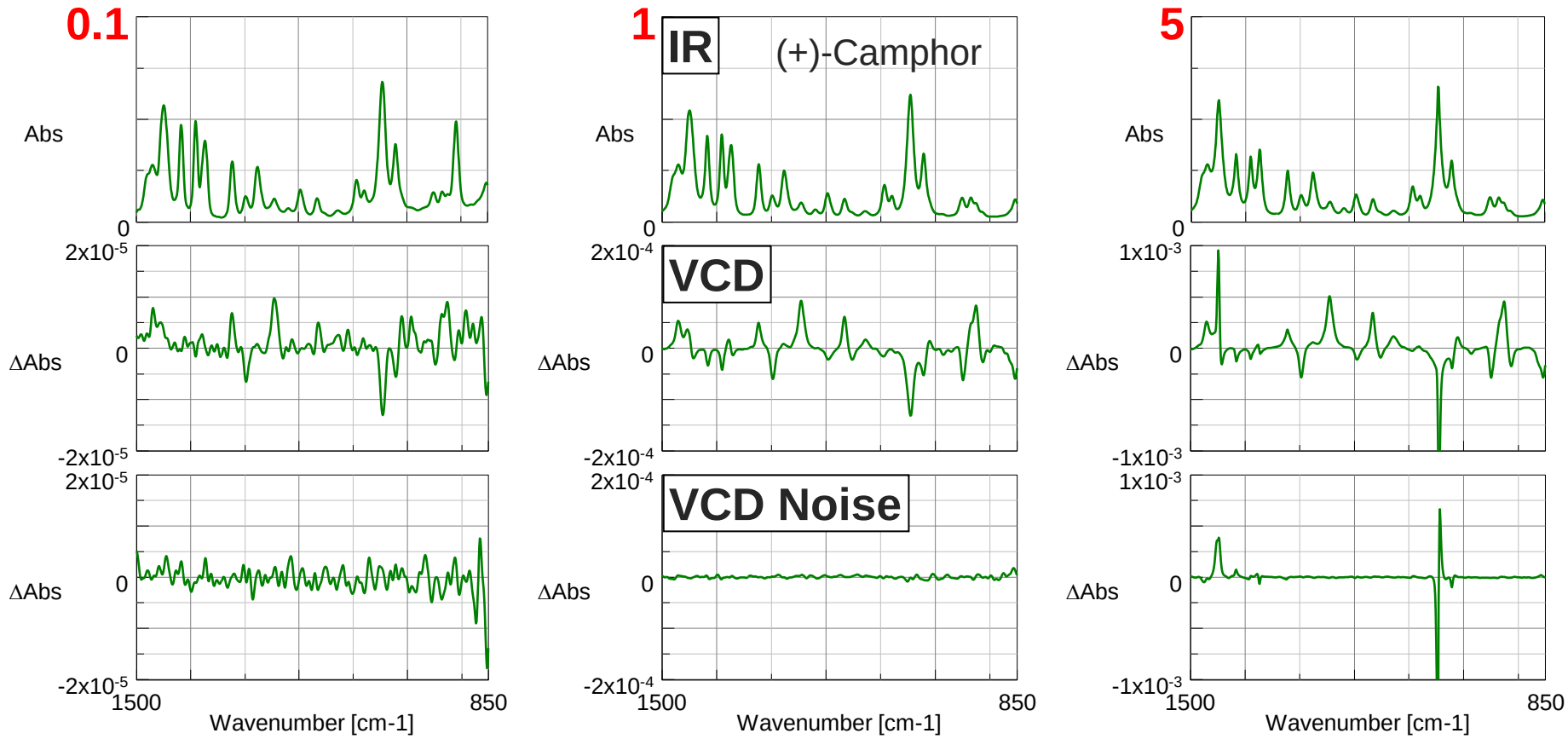


2. Shortening the optical path length reduces solvent absorption

However, note that a higher concentration sample is required

Sample Concentration Adjustment

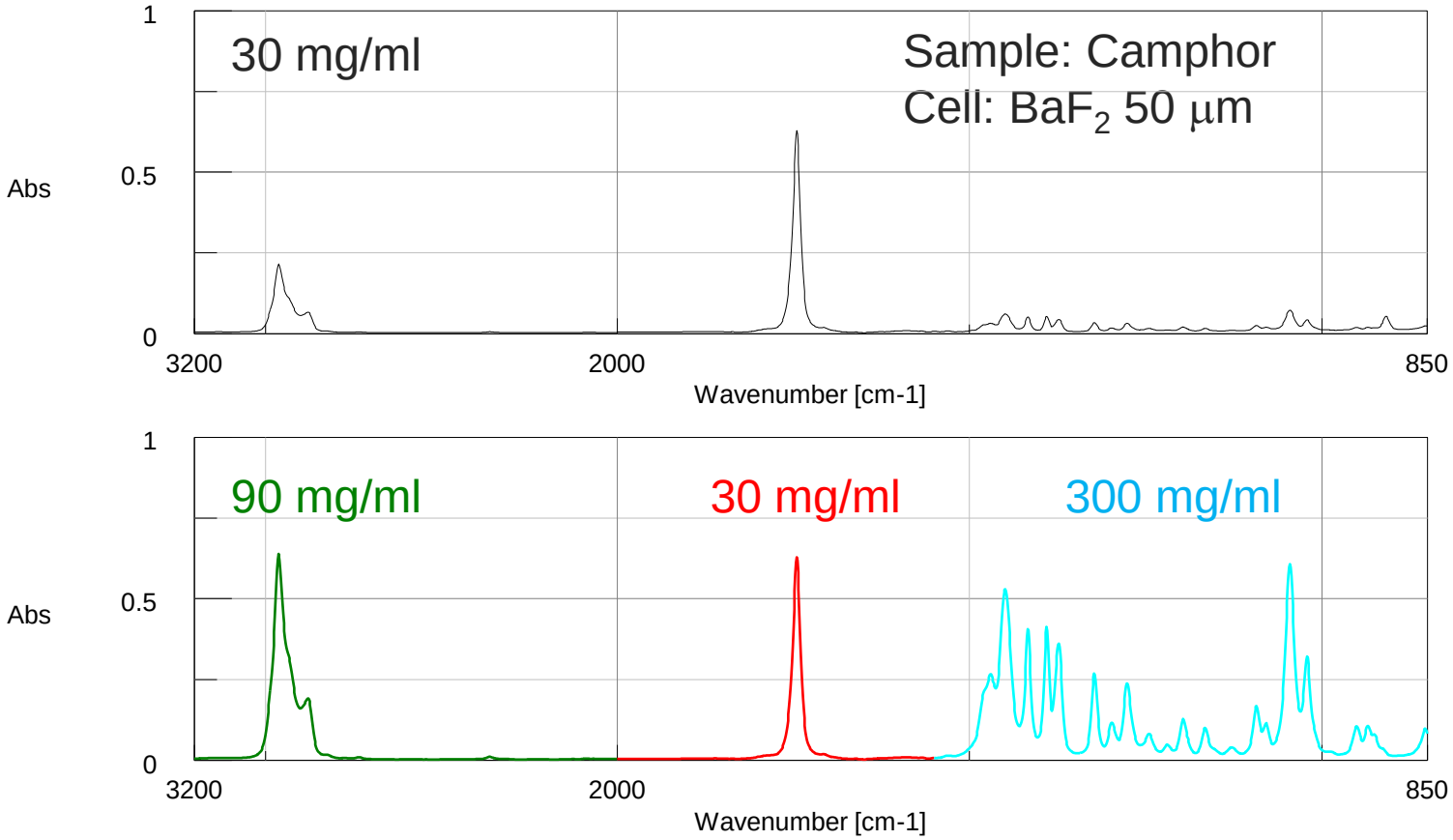
Concentration dependence for VCD spectra



Low concentration | Most appropriate | High concentration

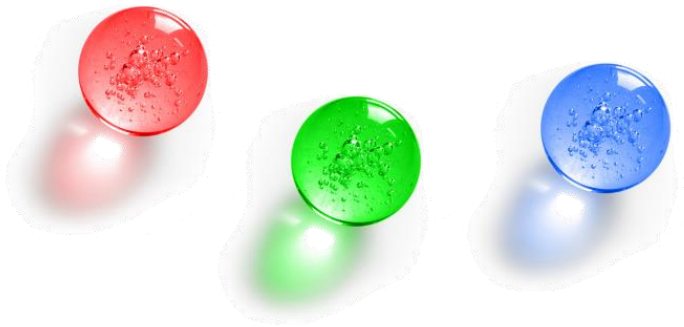
High quality VCD spectrum can be obtained when IR absorbance is adjusted to approximately 0.7.

Sample Concentration Adjustment with Wavenumber



If the absorbance for a sample differs greatly for different target peaks, when measurements are performed on samples with the most appropriate concentration for each peak, high-quality VCD spectra can be obtained.

Contents

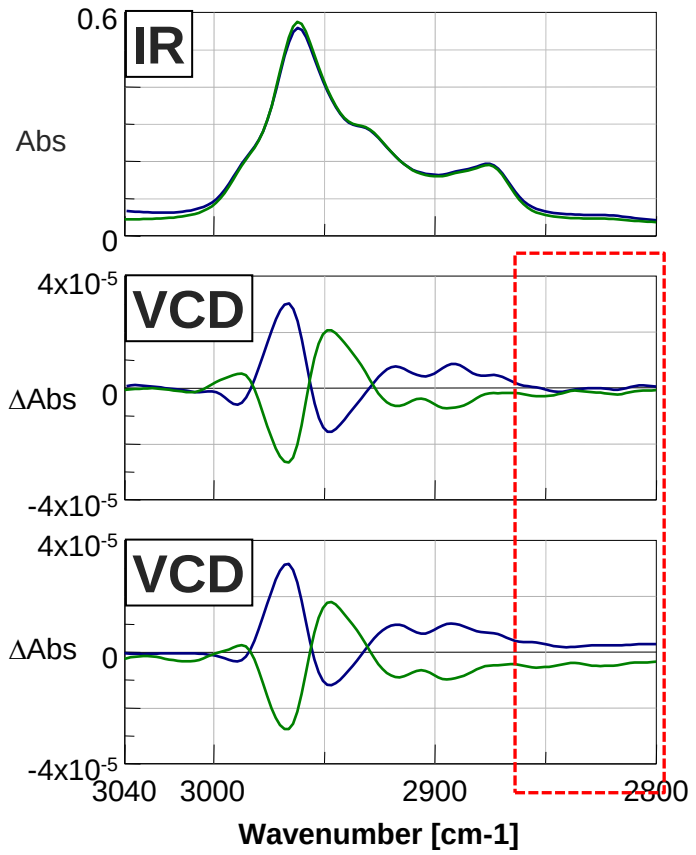


Measurement Examples using Autosampler

Autosampler (TAS-FVS)

- Up to 3 samples such as D-form, L-form and solvents can be measured sequentially.
- The influence of baseline drift with time can be reduced by changing a sample in a short time. (shuttle effect)

Shuttle Effect



With shuttle effect

（autosampler use）

Without shuttle effect

By changing a sample in a short time, the baseline of the VCD spectrum becomes close to zero due to the shuttle effect.

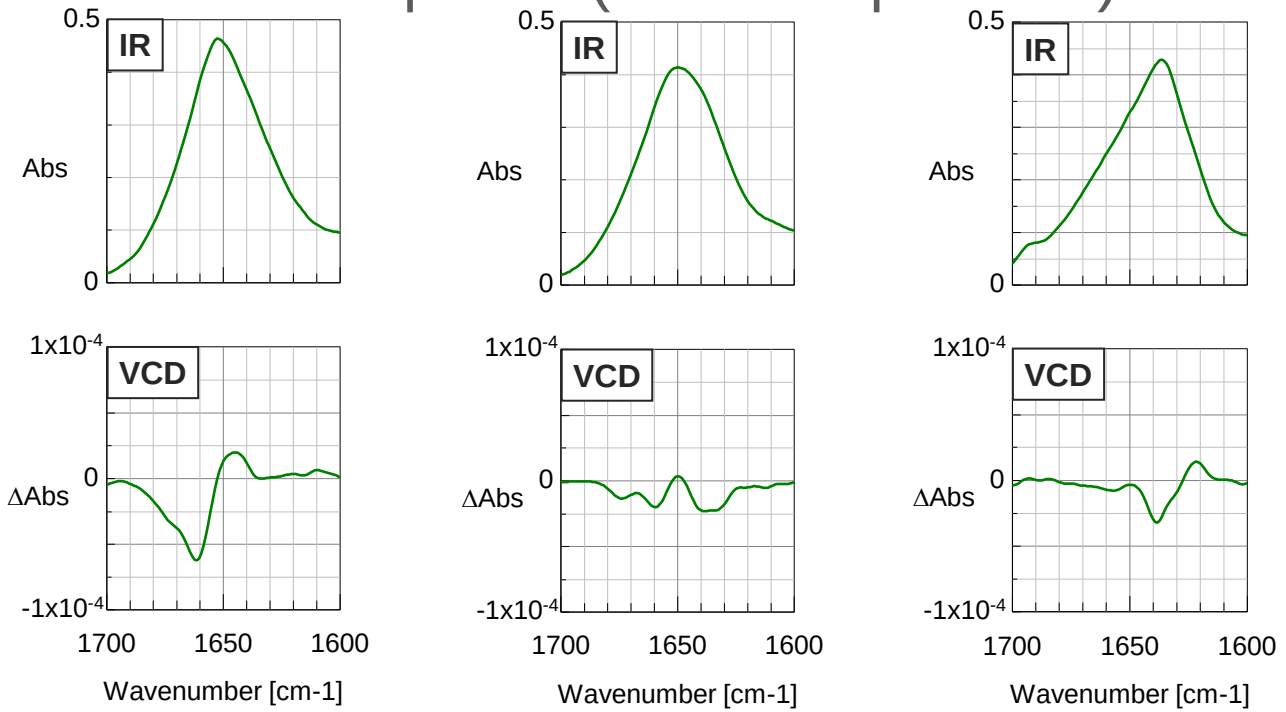
Measurement Example using Thermostatted Cell Holder

Thermostatted cell holder (TCH-FVS)

- Spectral change with temperature can be obtained from -5 to 90°C

Measurement Examples for Proteins

Protein spectra (room temperature)



Hemoglobin

Lysozyme

Concanavalin

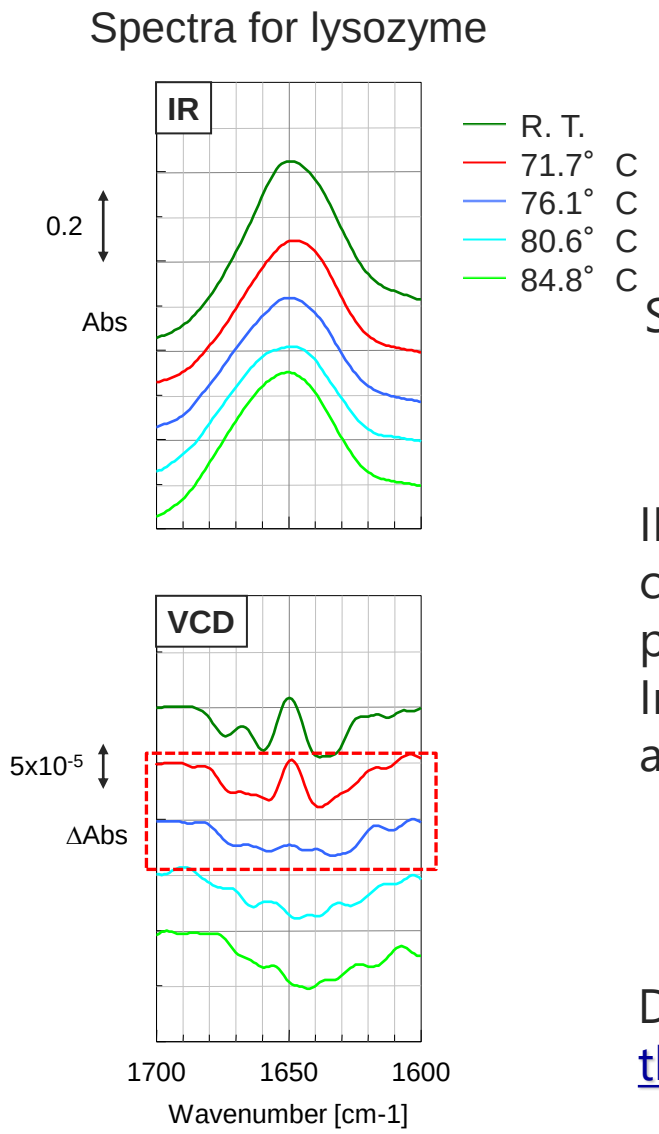
α- Helix rich

Mixture of α- helix and β-sheet

β- Sheet rich

Secondary structure of proteins can be evaluated based on VCD spectra

Evaluation of Thermal Denaturation of Lysozyme



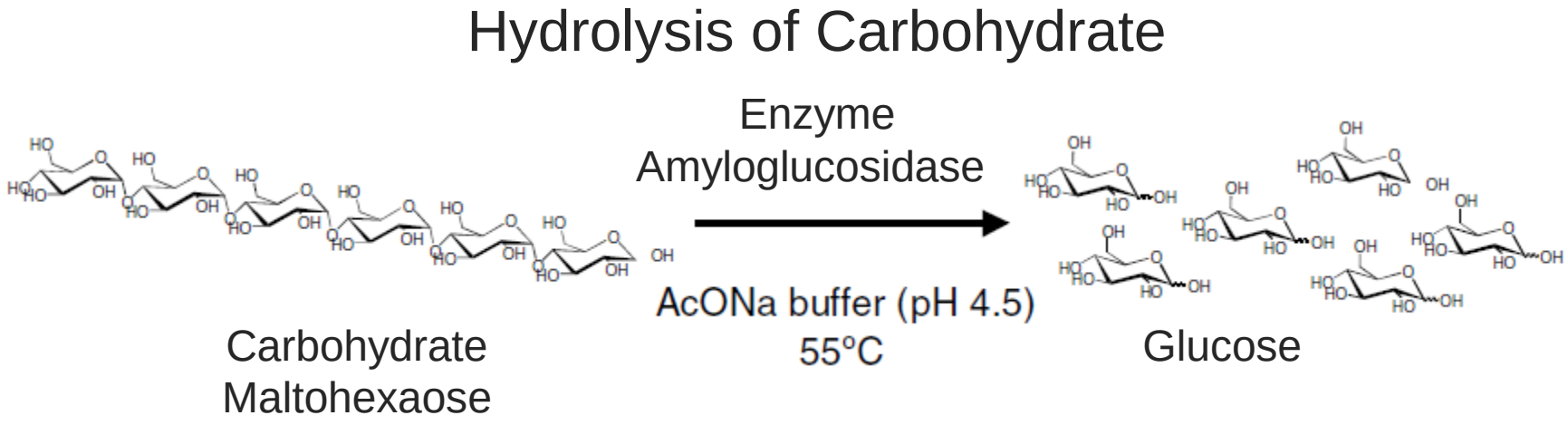
IR spectrum shape does not greatly change, but VCD spectrum shape changes starting at 76.1 °C

Suggests protein denaturation between 71.7 and 76.1 °C

IR peak near 1650 cm^{-1} associated with α-helix or random coil, but difficult to distinguish higher order structure of proteins.
In VCD spectrum, negative peak due to α-helix observed at wavenumber higher than 1650 cm^{-1}.

Disappearance of peaks derived from α-helix means that the α-helix structure has been lost by heating lysozyme.

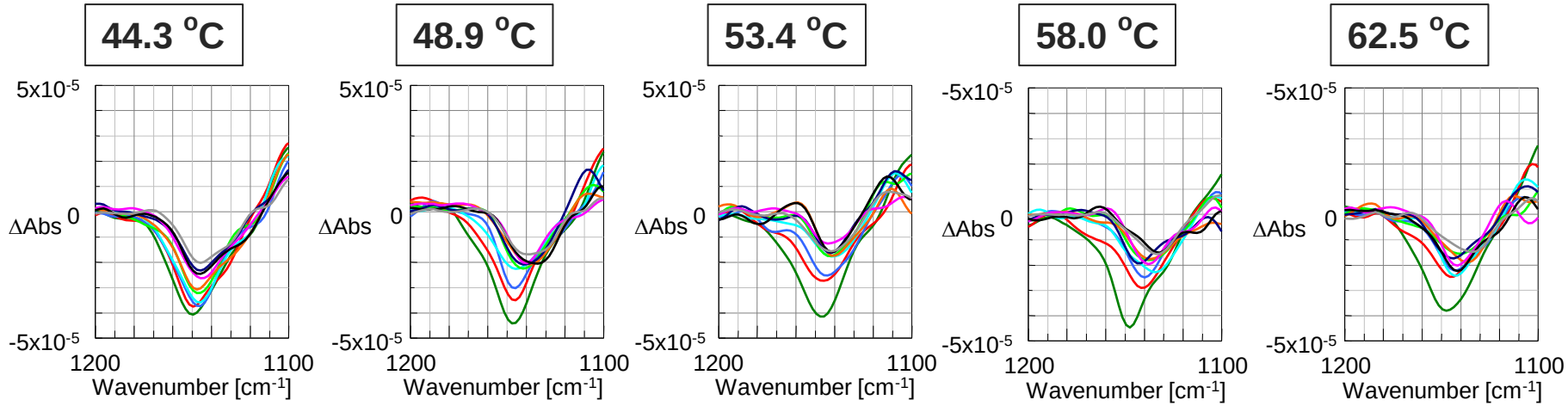
Temperature Dependence of Enzymatic Carbohydrate Reaction



- When maltohexaose is hydrolyzed to glucose, the glucosidic bond in the carbohydrate weakens.
- Since the VCD spectrum of carbohydrate contains a negative peak at 1149 cm^{-1} associated with glucosidic bonds, it is possible to analyze the enzyme reaction in real time by monitoring the intensity of this peak.

K. Monde, T. Taniguchi, N.Miura, S. Nishimura, Specific Band Observed in VCD Predicts the Anomeric Configuration of Carbohydrates, *J. Am. Chem. Soc.*, **2004**, *126* (31), pp 9496–9497

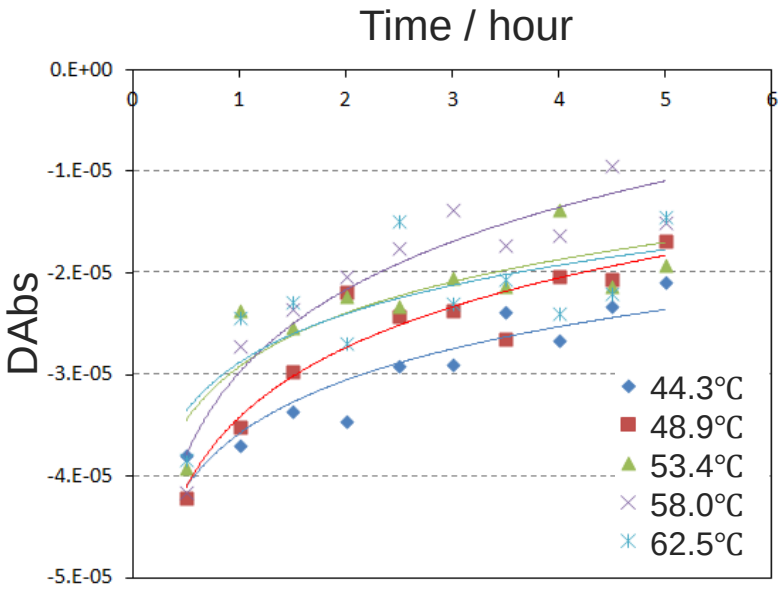
Temperature Dependence of Enzymatic Reaction



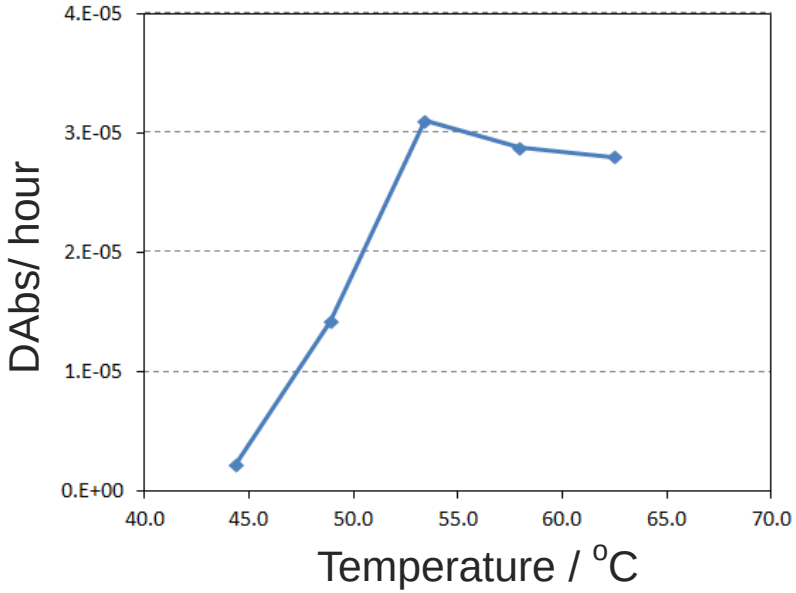
Temporal change in the VCD spectrum for maltohexaose (0.5 M) and amyloglucosidase 6.0 units/ml)

The change in peak height with time depends on the temperature

Temperature Dependence of Enzymatic Reaction



Temporal change in intensity of VCD peak due to glucosidic bonds at different temperatures



Change in the initial enzyme velocity with temperature

Change in glycosidic bonds by enzymatic reaction is temperature dependent

Optimum temperature for amyloglucosidase activity is expected to be 55 to 60 °C

Conformation for Carnitine Family

Special Issue Article

CHIRALITY 27:907–913 (2015)

Vibrational Circular Dichroism (VCD) Reveals Subtle Conformational Aspects and Intermolecular Interactions in the Carnitine Family†

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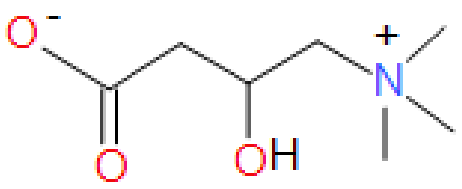
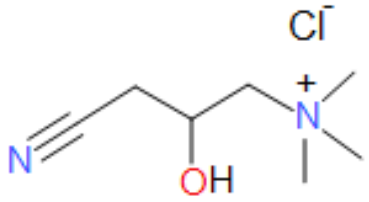


Carnitine

L-Carnitine: Nutrients used for lipid metabolism. Used for diet foods and food additives

D-Carnitine: toxic

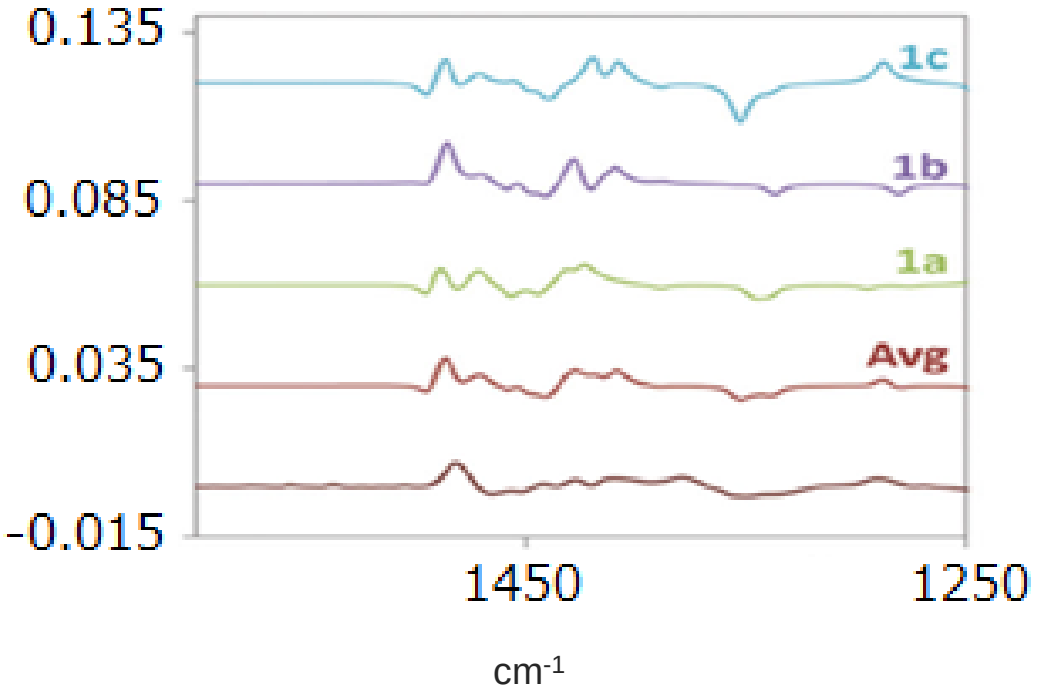


Carnitinenitrile chloride

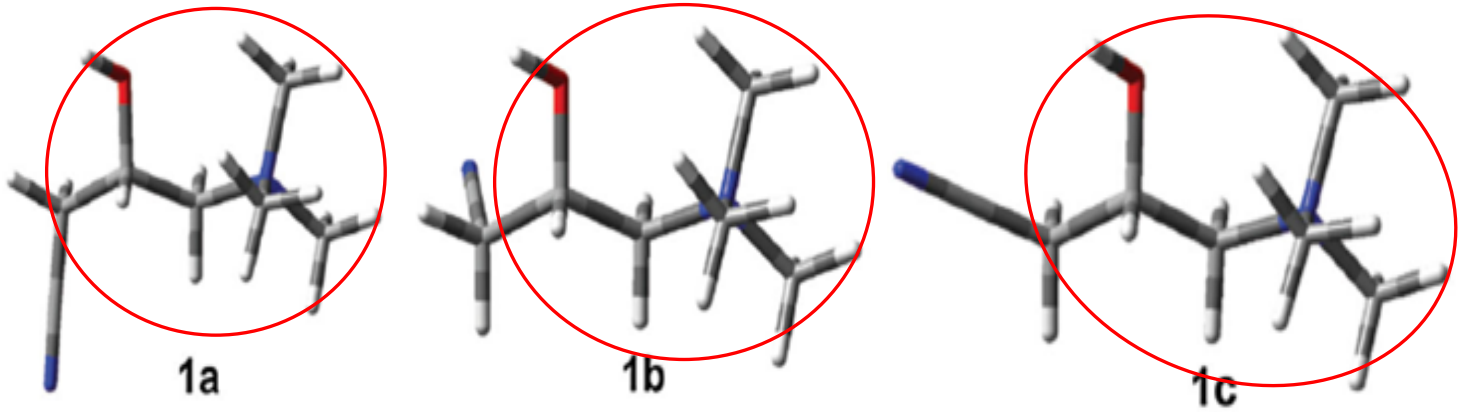
- Synthesis of enantiopure carnitine and analysis of excess enantiomeric percentage are important
- Analysis of carnitine by ECD and OR has been reported, but there are almost no examples of VCD measurements that obtained useful information
- VCD measurement of carnitine family and conformation estimation by calculation

Conformation of Carnitine Family

Comparison of experimental and calculated VCD spectra



Most common conformers



Since the information obtained from the VCD spectrum is abundant, in addition to chirality recognition, determination of absolute configuration and examination of conformational characteristics can be performed.

Summary

1. Basic principles

• No chromophores required because all organic compounds absorb in the IR region

• The absolute configuration of a sample can be determined by comparing the measured spectrum with that obtained using molecular orbital calculations

Summary

2. Instrumentation

•Approaches for measuring weak VCD signals using FVS-6000

Use a high-speed, and high-sensitivity liquid nitrogen cooled detector, and limit the measured wavenumber range using an optical filter

Summary

3. Measurement procedures

•The solubility should be checked in advance due to the high sample concentration

•It is desirable to select a solvent with low absorption, and in some cases it is necessary to use a deuterated solvent or to adjust the optical path length

•The sample concentration should be adjusted so that the absorbance in the IR region is approximately 0.7

Summary

4. Applications

•When using an autosampler, VCD spectra with a flat baseline can be obtained by the shuttle effect

•Various applications involving heating measurements were described

•Molecular conformation analyses based on the VCD spectrum were introduced

JASCO Educational Resources

Upcoming Webinars:

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- FTIR Theory, Instrumentation, and Techniques
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- SFC Theory and Applications

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- Raman
- Fluorescence
- FTIR
- CD

KnowledgeBase

ResearchGate

- Fundamental theory and application of circular dichroism spectroscopy

NEXT WEBINAR WILL BE ON
Circular Dichroism Part 2

Dr. Leah Pandiscia
TUESDAY APRIL 28TH AT 2:00 PM EDT

Thanks for joining us!!
Questions?