Application Note

Analysis of crystal polymorphism by Raman Spectroscopy for Medicine Development

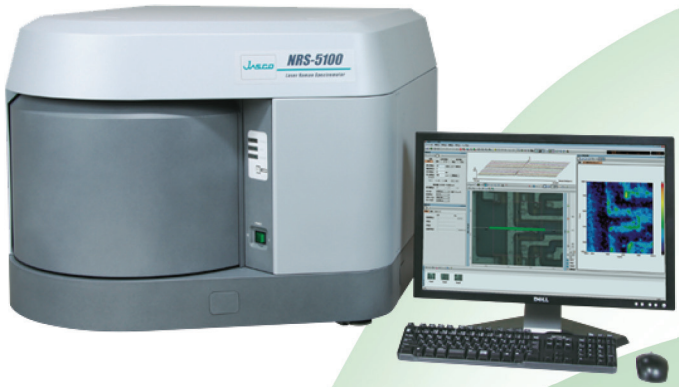
Introduction

Crystal polymorphism is a phenomenon where compounds with the same chemical formula can be crystallized into different crystal structures. The crystal structure depends on the conditions (such as temperature and pressure) used in the crystal formation process. The resulting crystal structures are conveniently classified as Form I, II, III or Ta and Tb.

In medicine, the solubility, stability and bio-availability will depend on the crystal polymorphism; compounds with different crystal structures may be recognized as different compounds. Therefore, research into crystal polymorphism is widely used in areas such as:- basic study, structure/function correlation of compounds and industrial applications such as patent application.

Therefore, in the pharmaceutical industry, the study of crystal formation techniques is very important for the control of crystal structure and the manufacture of high quality medicines.

Raman applications in the pharmaceutical Industry



Micro-Raman spectrometer (NRS-5000)

- Impurity Analysis (Identification of micro impurity with sizes less than 1 um)
- Evaluation of Synthetic (Structural analysis in combination with IR)
- Mapping (Evaluation of tablet for DDS research)
- PAT (Real-time control in medicine production process)

Evaluation of Crystal Polymorphism (Evaluation method instead of XRD and DSC)

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Method for evaluation of major crystal polymorphism

Three analytical techniques are used in the evaluation of crystal polymorphism

	Principle	Advantage	Disadvantage
XRD	Evaluation of crystal structure by X-ray diffraction.	Accurate distance between atoms in crystals. XRD is widely used for crystal structure analysis.	Amorphous structure cannot be evaluated by XRD.
DSC	Stability and melting point of crystals are evaluated by endothermic/exothermic reaction of the crystal when heating/cooling the sample	Properties of the crystal can be observed.	Crystal structure cannot be determined by DSC.
Raman	Chemical structure is evaluated by detecting scattering light at low wavenumbers derived from lattice vibration, by laser irradiation of the sample.	Both crystal and amorphous structures can be evaluated. Vibrational information of molecules can also be obtained from high wavenumber spectra . High spatial resolution and micro measurement can be done. Sample preparation is not required.	It is difficult for Raman to evaluate the absolute configuration of a crystal.

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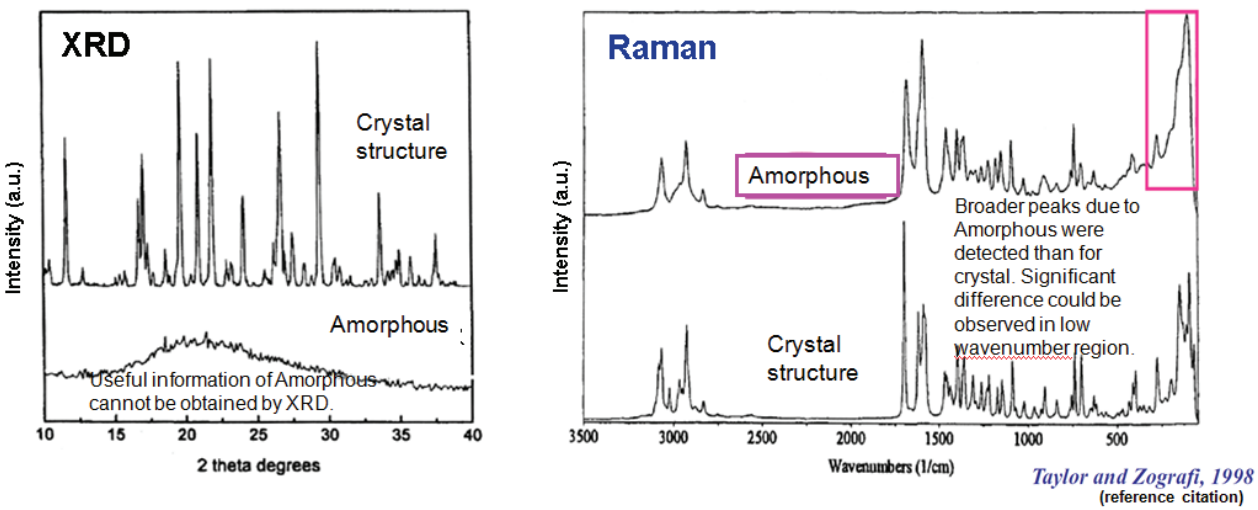
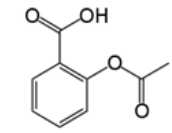
Features of Raman Spectroscopy for evaluating Crystal Polymorphism (v.s. XRD)

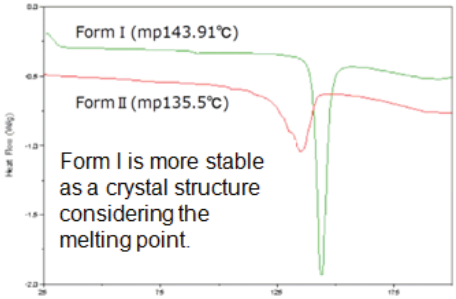


The above spectra show measurement results for both Crystal and Amorphous structures of Indometacin using XRD and Raman spectroscopy. Generally it is difficult to measure samples with less reflection (less diffraction spot) such as Amorphous and minute crystals using X-ray crystal analysis. Since the actual medicines include Amorphous and minute crystals in a mixture, Raman Spectroscopy is more effective as shown on the right Figure. Presently X-Ray crystal analysis is used for the basic study of crystal structure for patent registration, however due to the above reasons, Raman is expected to become a technique more widely used in the pharmaceutical industries for patent registration.

Features of Raman spectroscopy in Crystal Polymorph evaluation (v.s. DSC)

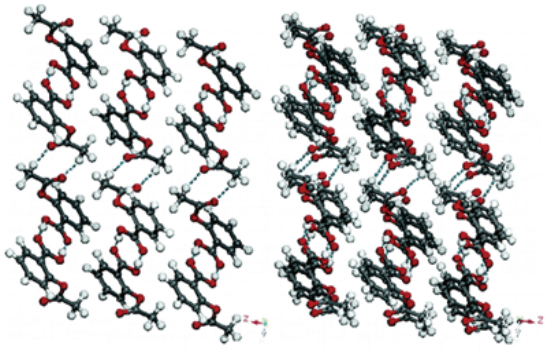


Acetylsalicylic acid (Aspirin)
A typical antiphlogistic analgesic and representing non-steroidal anti-inflammatory medicine.



Measurement result of acetylsalicylic acid using DSC (cited from reference paper)

Journal of the American Chemical Society
127(2005),16802-16803



Form I Form II

It is known that Acetylsalicylic acid can exists in crystalline Form I and Form II. XRD can be used for crystal structure analysis, but the thermal stability cannot be evaluated and useful spectra cannot be obtained for Amorphous structures at temperatures higher than the melting point. Conversely, DSC can be used to evaluate the thermal stability of a crystal, but structural information cannot be obtained.

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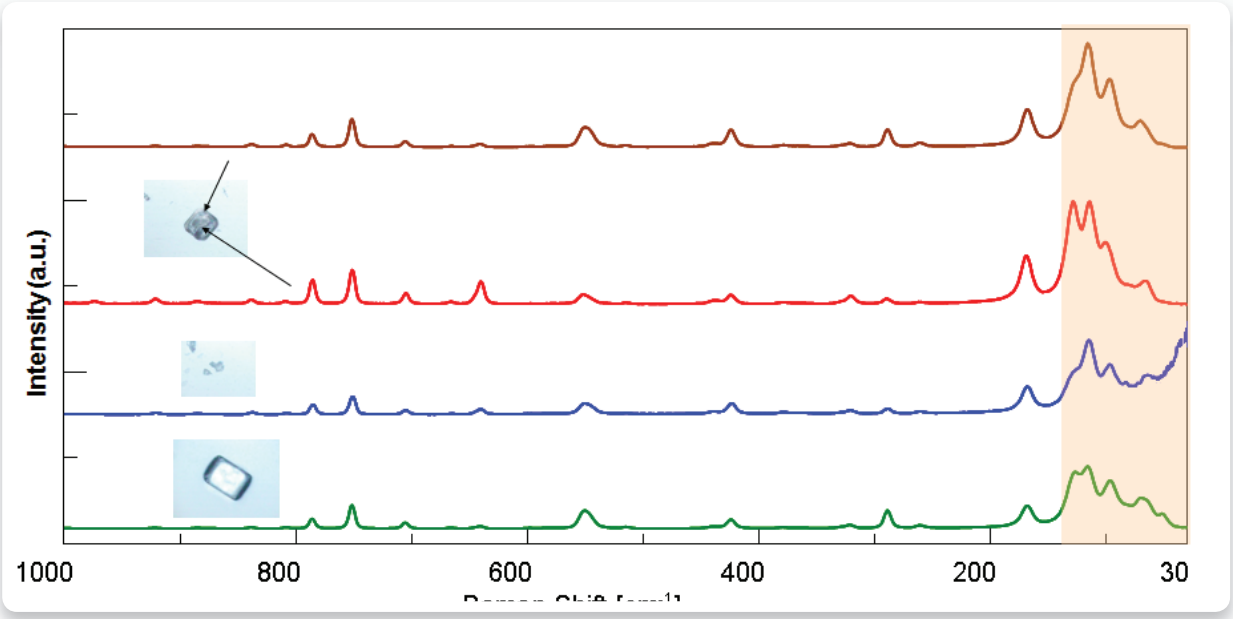
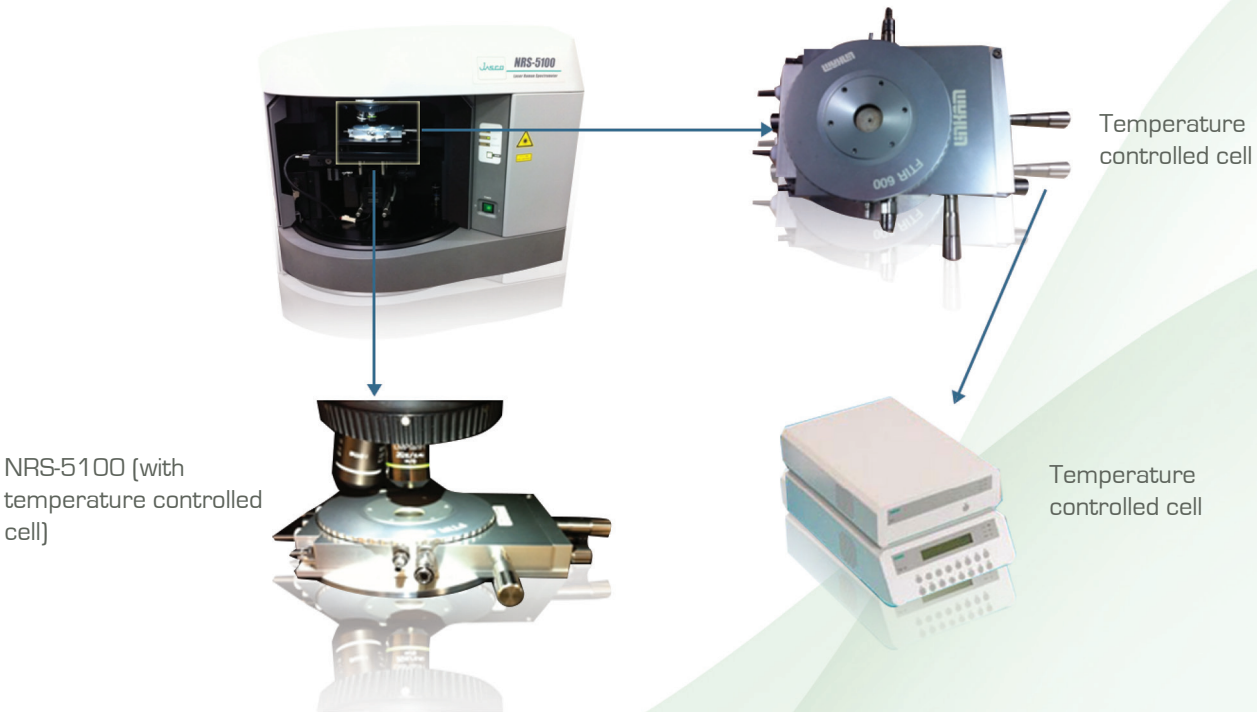
Raman spectra of Acetylsalicylic acid of different particle size



Commercial Acetylsalicylic acid with several different particle sizes were selected and measured using Raman. The results show a change in peak ratio observed in the higher wavenumber region, this is due to the differences in the crystal axis. In addition, difference in the Raman spectra was observed in the region below 200 cm-1 due to difference in particle size and measured position. These results indicates that the commercial medicine is in a mixed crystal state and it is therefore difficult to analyze using DSC due to the different melting points of the crystal forms.

NRS-5000 system with heating stage



By mounting temperature control system on NRS-5000 series, it is possible to monitor in-situ the structural changes in samples.

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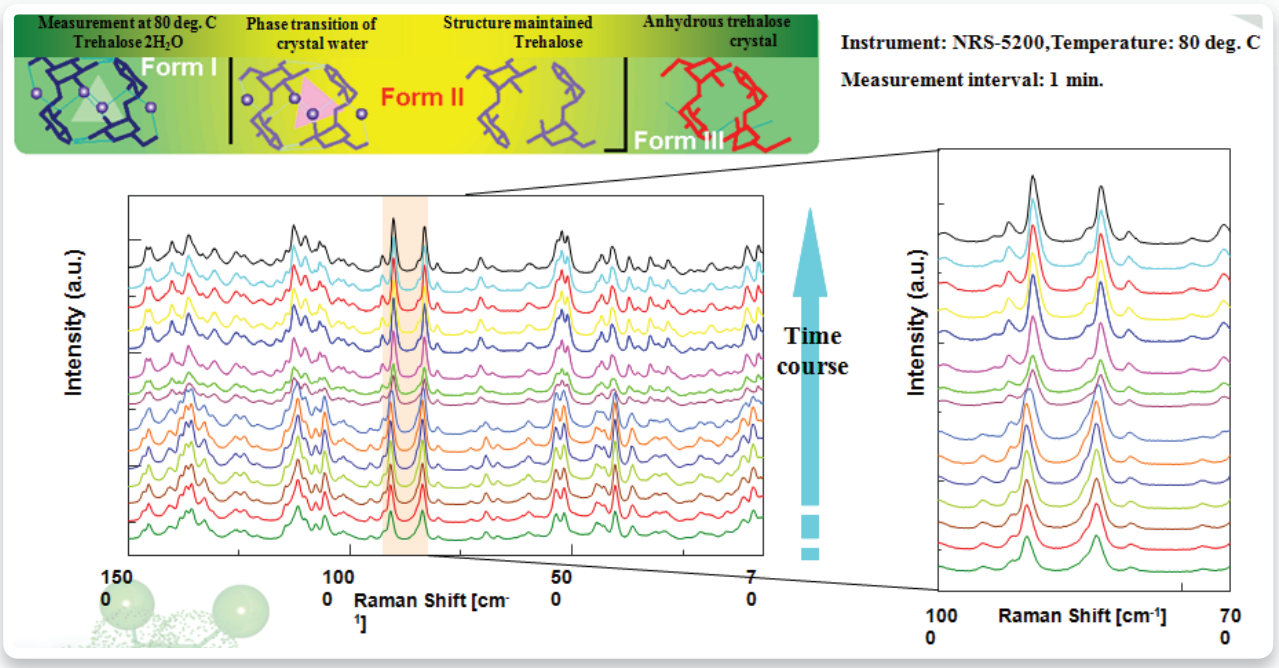
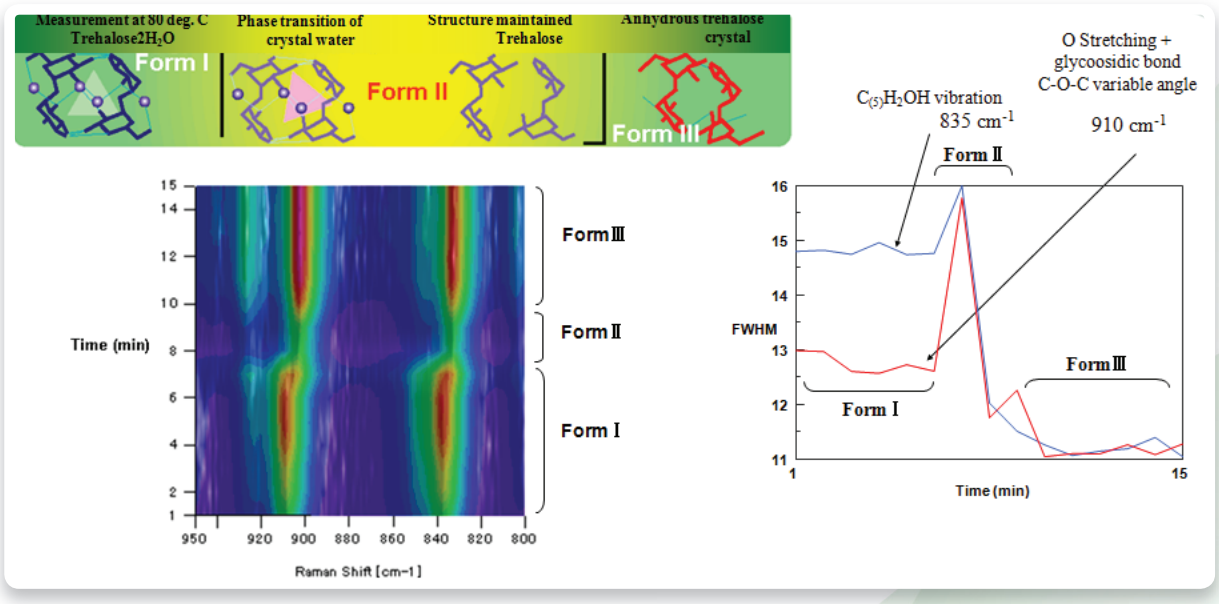
Real time monitoring of Trehalose using heating stage 1



In-situ monitoring of Trehalose 2H2O (which has high water retention and is used for foods and medicines) at 80 deg. C - spectra measured at one minute intervals. The results shows time-dependent changes in the spectra.

Real-time monitoring of Trehalose using heating stage 2



Half bandwidth and the peak shift of two peaks which demonstrates the hydration feature in the previous page were analyzed. The result indicates that the changing process of the peak shift and half bandwidth was observed in three steps and by analyzing peaks in the region 1000-700 cm-1 it was confirmed that the crystal structure of Trehalose changes from a pre-stable structure (crystal or Amorphous) to a stable structure. The combination of Raman fitted with a temperature control system enables the analysis of kinetic structural change with sample melting.

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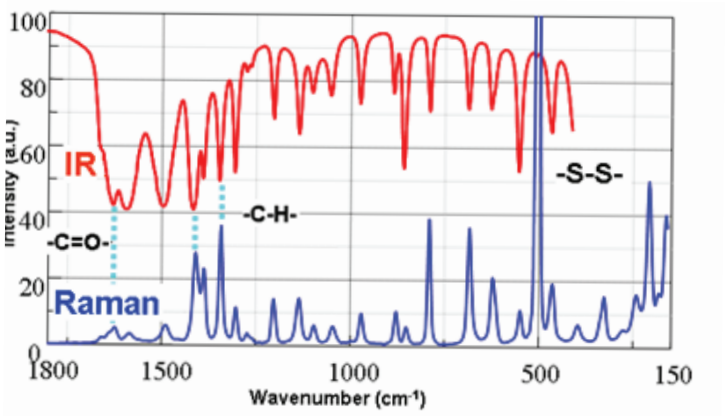
Features of the NRS-5200 in the evaluation of crystal polymorphism



IR and Raman spectra
(L-cystine[$-SCH_2CH(NH_2)CO_2H]_2$)

Micro-Raman spectrometer (NRS-5000)

- High spatial resolution
- Lattice vibration
  (very low wavenumber measurement)
- No sample pretreatment

The above figure shows IR and Raman spectra of L-cystine. Raman spectroscopy enables measurement in the low wavenumber region below 400 cm-1, in addition, the NRS-5200 can provide information for the crystal lattice vibration in the very low wavenumber region down to 10 cm-1. Since Raman scattering from aqueous solution or glass is weak, sample pretreatment is not necessary and samples in glass or buried samples can be measured easily and non-destructively. One of the features of NRS-5000 is that crystal polymorphism can be evaluated quantitatively and qualitatively at a high spatial resolution down to 1 um.

Low wavenumber measurement using Notch filter

What is Notch filter...?

A Notch filter is an optical element used to remove the very strong scattering such as Rayleigh scattering light from the sample. Measurements of crystal lattice vibrations are made in the very low wavenumber region and the Notch filter and low wavenumber measurement unit are required to virtually eliminate Rayleigh scatter.

Notch filter angle can be adjusted by the user!

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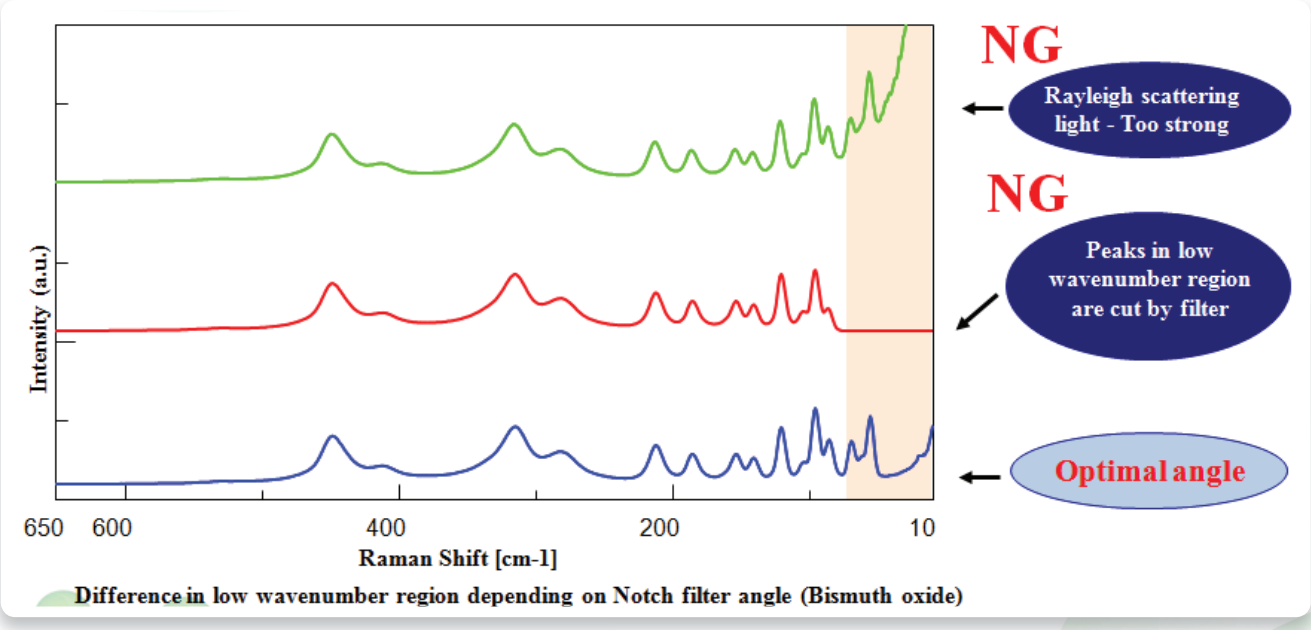
NRS-5000 series allows not only measurement of the usual inorganic and organic samples, but also measurement in the low wavenumber range using the most suitable optical arrangement. It is possible for the user to easily adjust the optimal angle of the Notch filter for low wavenumber measurement as required for each sample. In addition the NRS-7200 allows very low wavenumber measurement down to 10 cm-1 using the low wavenumber measurement unit.

NRS-5100/7100	down to 50 cm-1
NRS-5200 with low wavenumber measurement unit	down to 10 cm-1
NRS-7200 with low wavenumber measurement unit	down to 5 cm-1

Low wavenumber measurement made by adjusting the Notch filter angleBismuth oxide



Difference in low wavenumber region depending on Notch filter angle (Bismuth oxide)

Since peaks of minerals or lattice vibration derived from crystals are detected in low wavenumber region, Raman measurement in the low wavenumber region is very important. Rejection of Rayleigh scattering is the key point to detect peaks in low wavenumber region, so that for careful optimization it is necessary to adjust angle of Notch filter depending on the sample requirements.

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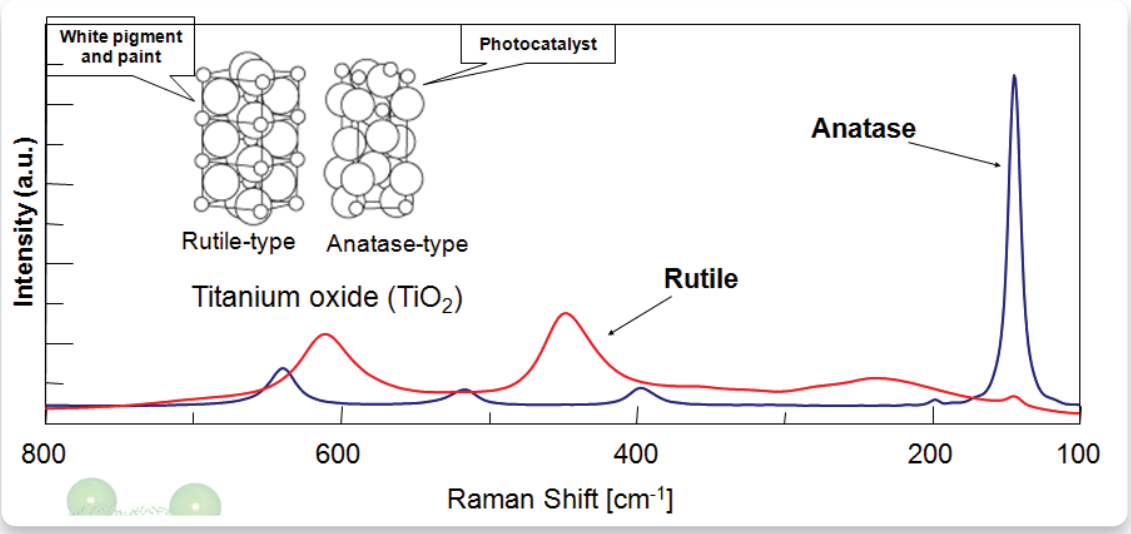
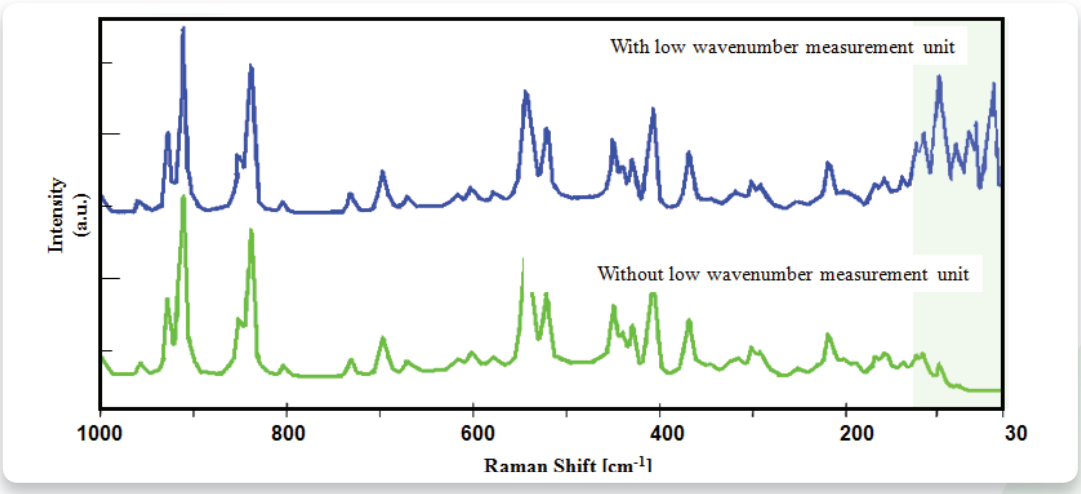
Low wavenumber measurement using Notch filter -Titanium oxide with several crystal polymorphs-



The above shows Raman spectra measured using NRS-5200 of two different crystal structures of Titanium oxide which are used as medicine coating, pigment and photocatalyst. By adjusting the notch filter angle, peaks in low wavenumber region can be measured, demonstrating the differences between Rutile and Anatase.

Measurement using low wavenumber measurement unit (NRS-7200) -Trehalose-



NRS-7200 with low wavenumber measurement unit

Raman spectra of Trehalose

Sometimes important peaks are cut by the notch filter when used with a with single monochromator, however the low wavenumber measurement unit fitted to the NRS-7200 enables clear measurement in the low wavenumber region.

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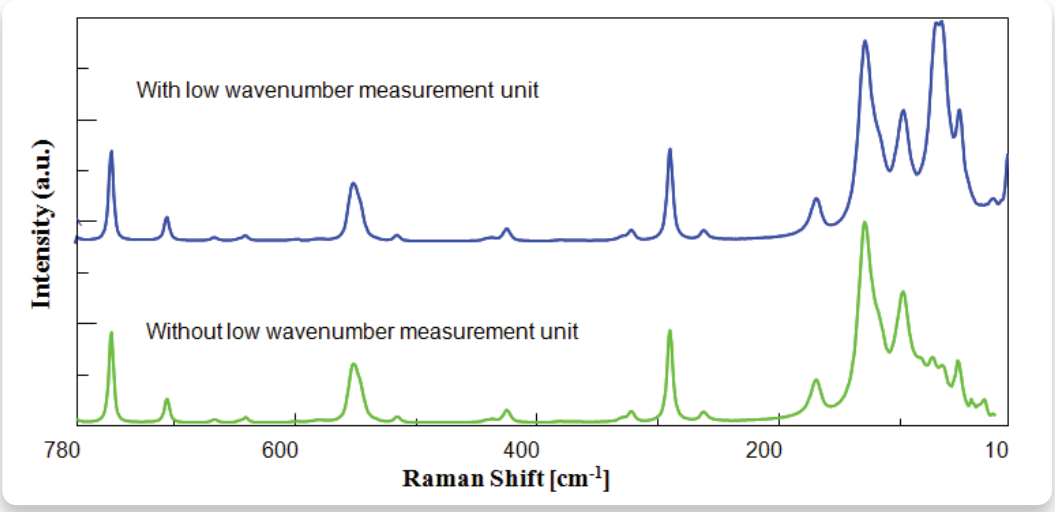
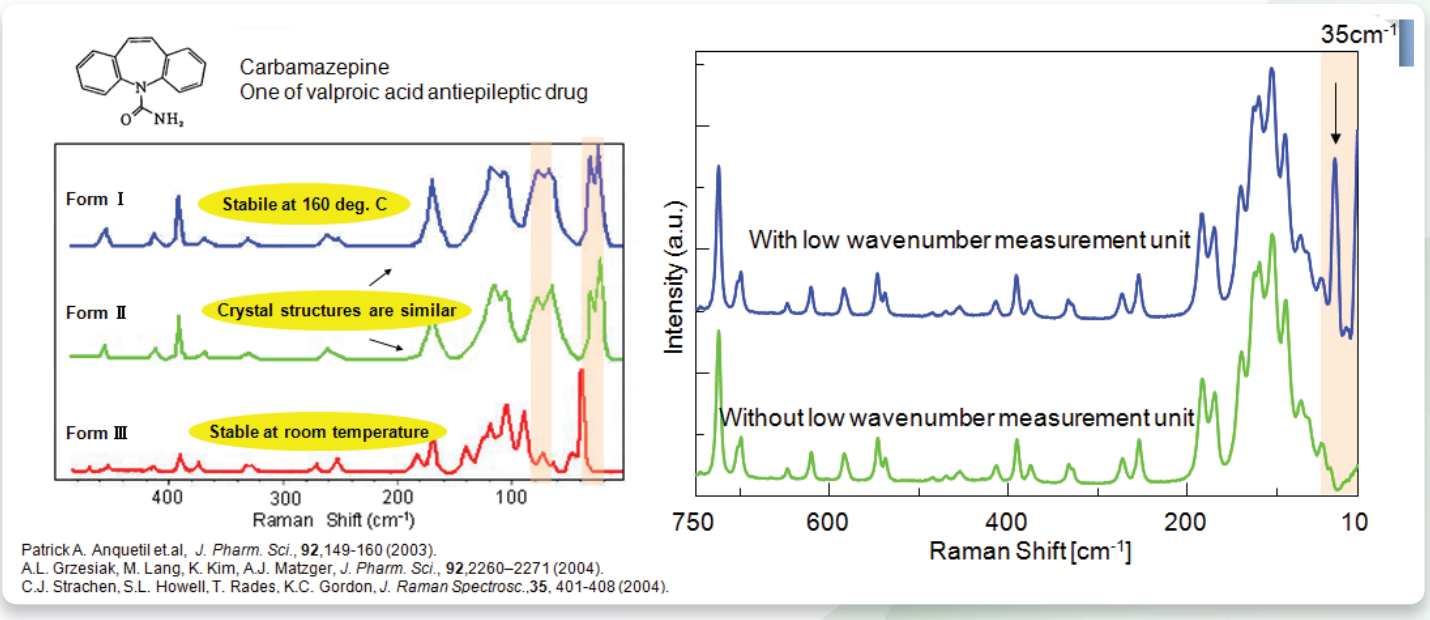
Measurement using the NRS-7200 fitted with a low wavenumber measurement unit -Acetylsalicylic acid-



Commercially available Acetylsalicylic acid was measured with the NRS-7200. The upper spectrum was obtained using the NRS-7200 fitted with a low wavenumber measurement unit, the lower spectrum, without the option. Using the low wavenumber measurement unit, peaks for the lattice vibration can be clearly observed and important information for crystal polymorph identification can be obtained.

Measurement using low wavenumber measurement unit (NRS-7200) -Carbamazepine which has several polymorphs-



Spectra of carbamazepine (cited from reference paper)

Spectra of carbamazepine (Form III) measured by

Since Raman can detect peaks in the wavenumber region below 100 cm-1, it is possible to identify the differences between Forms I and II of carbamazepine whose crystal structures are quite similar.

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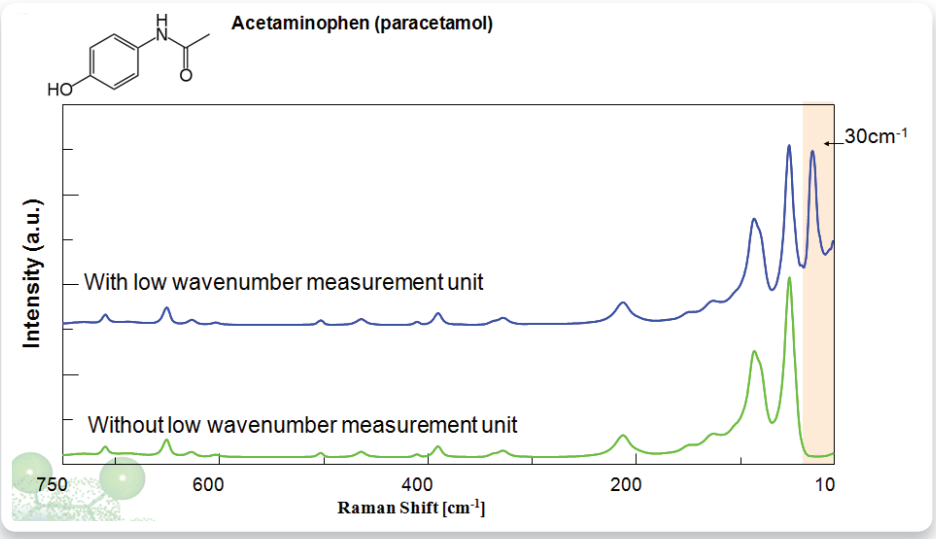
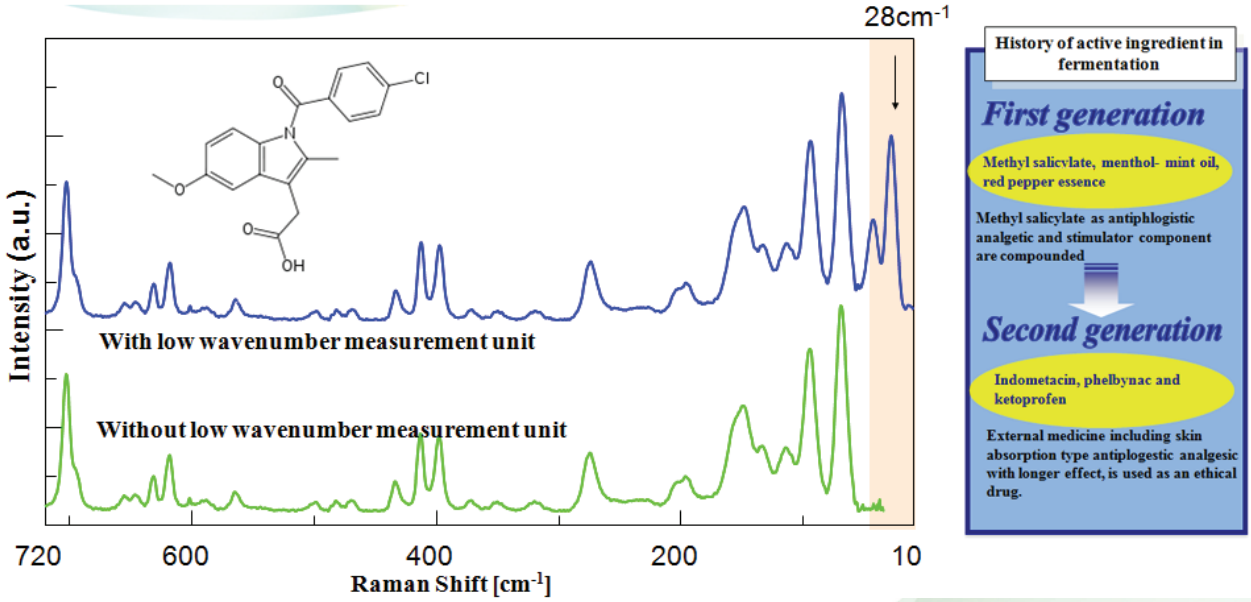
Measurement using low wavenumber measurement unit (NRS-7200) -Acetaminophen (paracetamol)-



Commercially available acetaminophen was measured using the NRS-7200. It is known that there are three crystal polymorphs of paracetamol - Form I, II and III. The above spectra shows Form I.

Measurement using low wavenumber measurement unit (NRS-7200) -patent of indometacin depending on crystal polymorphism-



The above spectra were obtained by measuring commercially available indometacine using NRS-7200 with and without the low wavenumber measurement unit. It is known that Indometacin exists as three different crystal polymorph:- alpha (acicular crystal), beta and gamma (plate crystal). Gamma is the most stable structure followed by alpha. Recently, alpha has been selectively manufactured (and patented) to be used for external medicine for enhanced skin absorption and stability. The method for effective manufacturing of a specified crystal polymorph is extremely important.

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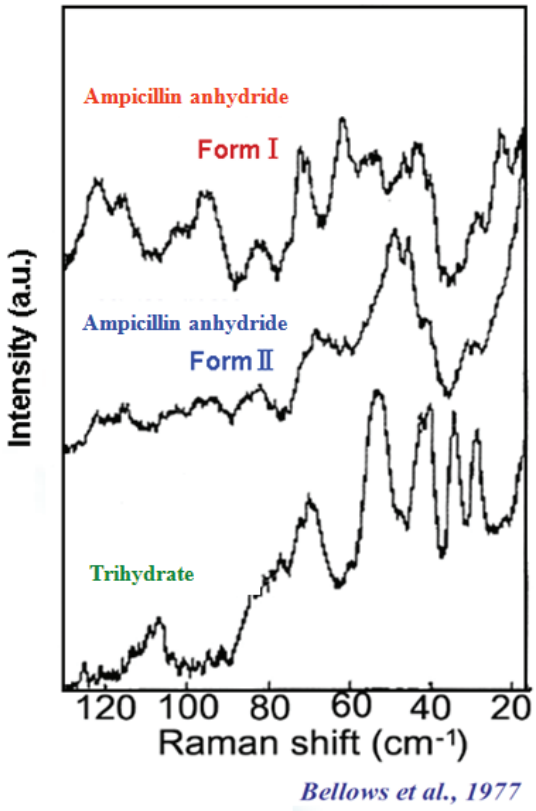
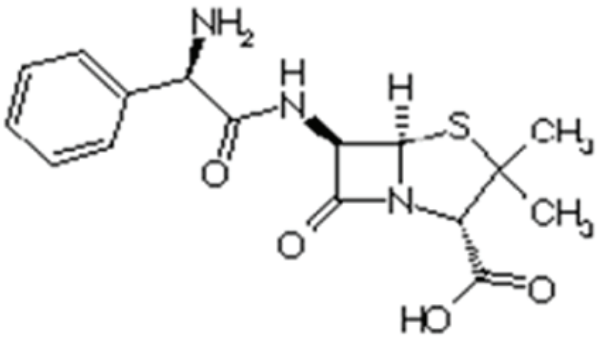
Research papers including low wavenumber measurement- Ampicillin which has several polymorphs-



Bellows et al., 1977

Ampicillin **A beta-lactam antibiotic**



Spectra on the left show crystal lattice vibration of ampicillin detected by Raman spectroscopy in the very low wavenumber region. It is possible to identify anhydride Form I, anhydride Form II and trihydrate in ampicillin from their Raman spectra.

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Conclusion of Raman and crystal polymorph

Comparing with XRD and DSC

Raman enables …

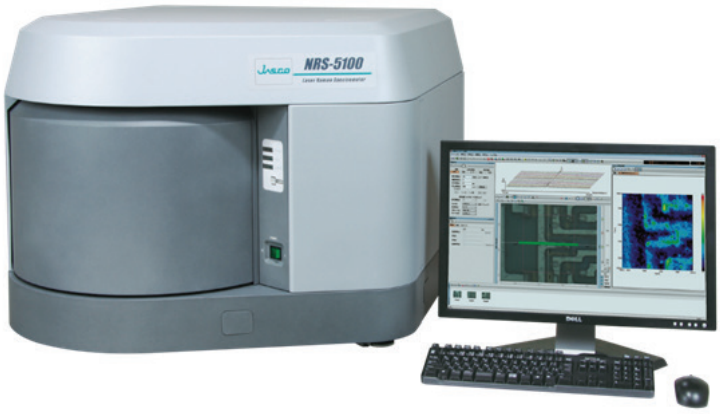
- Measurement of amorphous structures
- Measurement of micro samples
- Provides information regarding crystal and molecular structure

Raman can be used to measure directly commercially available medicine, suggesting the evaluation of final products

Low wavenumber measurement is essential for evaluation of crystal polymorph!

Comparing with other Raman instrumentsNRS-7200 allows …

- Measurement in low wavenumber range by angle adjustable notch filter
- Measurement in very low wavenumber range using Low wavenumber measurement unit



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