

## Raman Spectroscopy Analysis of Crystalline Polymorphs for Pharmaceutical Development

### Raman Applications in the Pharmaceutical Industry

- Impurity Analysis (Identification of micro impurities - sizes less than 1  $\mu\text{m}$ )
- Evaluation of Synthesized Compounds (Structural analysis in combination with IR)
- Mapping (Evaluation of tablets for DDS research)
- PAT (Real-time control in pharma production process)

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

**Keywords:** NRS-5000 , NRS-7000, dispersive Raman, Raman spectroscopy, Raman imaging, thermal melting, DSC, XRD, pharmaceutical analysis, Acetylsalicylic acid, aspirin, crystal polymorphism, crystal structure, trehalose, ampicilin, beta lactam, TiO<sub>2</sub>, Titanium dioxide, Indomethacin



Jasco NRS Series Raman Micro-spectrometers

**JASCO INC.**

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Application Library: <http://www.w.jascoinc.com/applications>

## What is a Crystal Polymorph ....?

Crystal polymorphism is a phenomenon where compounds with the same chemical formula can be crystallized into different crystalline 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 T<sub>a</sub> and T<sub>b</sub>.



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

**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.**

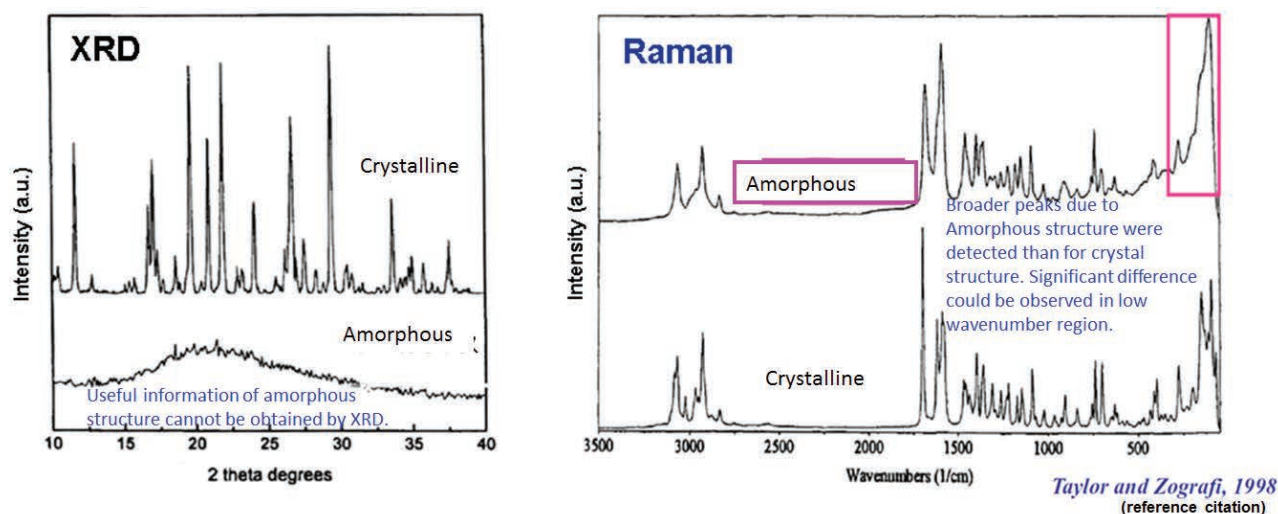
## Methods for Evaluation of Crystal Polymorphism

Three analytical techniques are used in the evaluation of crystal polymorphism

Instrument	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.

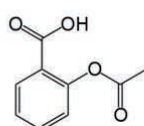
Raman Spectroscopy is one of the hot analytical techniques for the study of crystal polymorphism!

## Features of Raman Spectroscopy for Evaluating Crystal Polymorphism (vs. XRD)



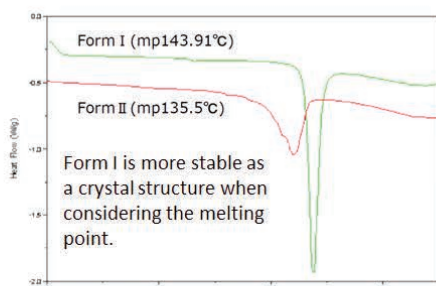
The above spectra show results for both crystalline and amorphous structures of Indometacin using XRD and Raman spectroscopy. Generally it is difficult to measure samples with less reflection (less diffraction spot) such as amorphous and/or minute crystals using X-ray crystal analysis. Since the actual medicine formulations include amorphous and minute crystals in a mixture, Raman spectroscopy is more effective as shown in 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 more widely used technique in the pharmaceutical industries for patent registration.

## Features of Raman Spectroscopy in Crystal Polymorph Evaluation (vs. DSC)

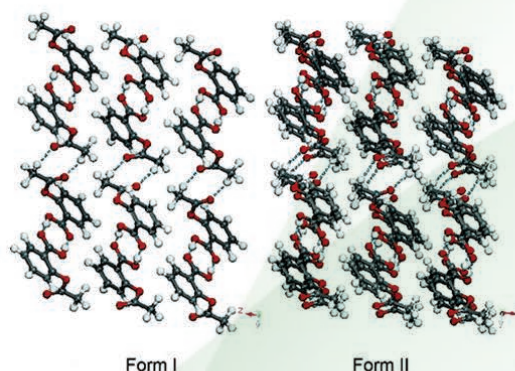


Acetylsalicylic acid (Aspirin)

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

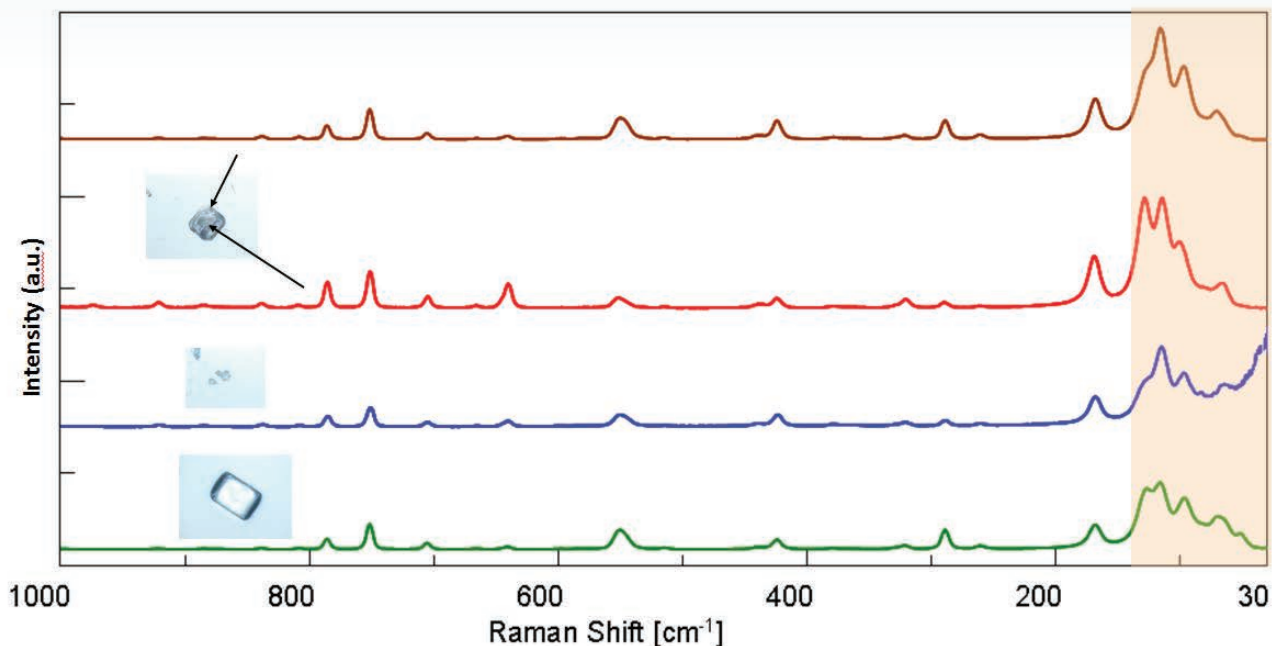


Measurement result of acetylsalicylic acid using DSC  
(cited from reference paper)



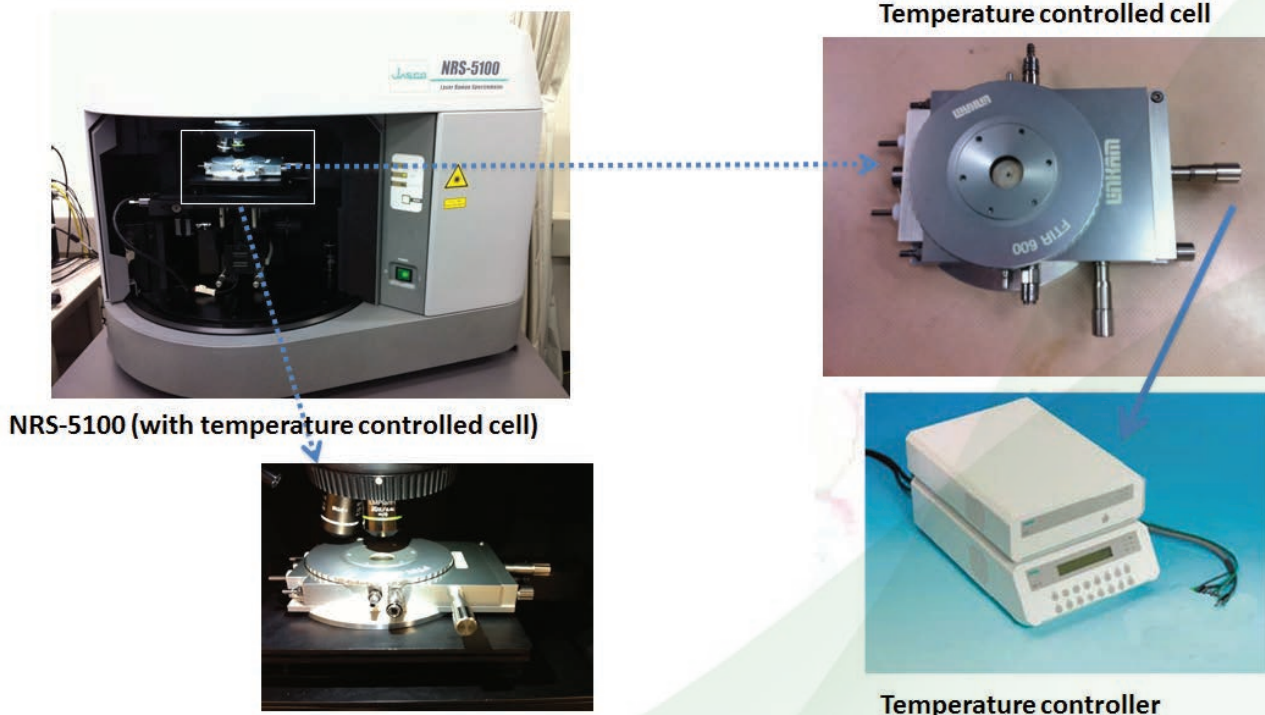
It is known that Acetylsalicylic acid can exist in crystalline Forms I and II. XRD can be used for crystal structure analysis, but thermal stability cannot be evaluated and useful spectra cannot be obtained for amorphous structures at temperatures higher than the melting point. Conversely, DSC can be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

## 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, differences in the Raman spectra were observed in the region below 200  $\text{cm}^{-1}$  due to differences in particle size and measured position. These results indicate 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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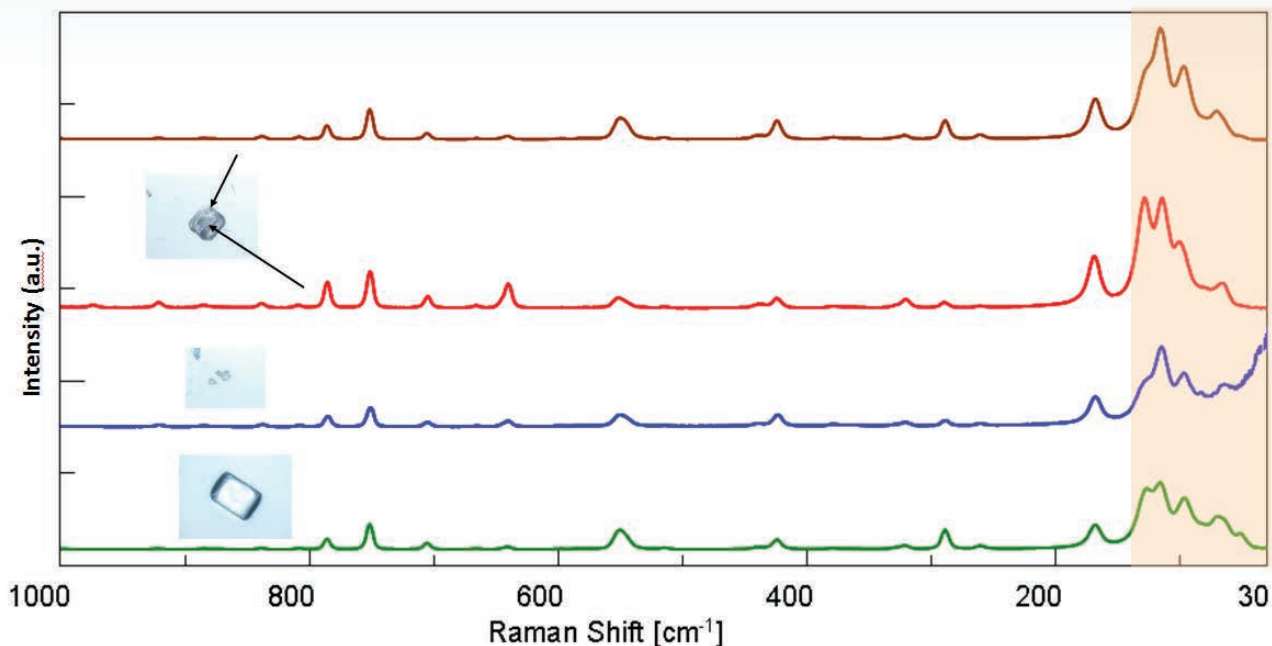
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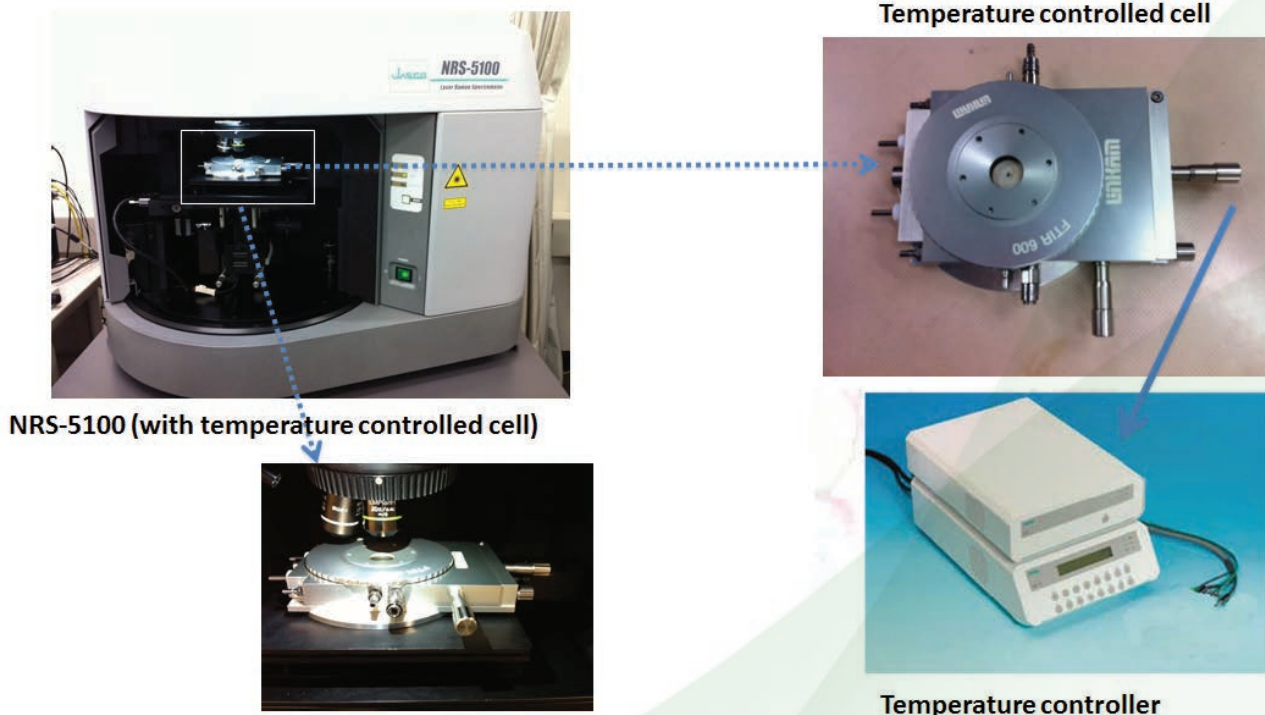
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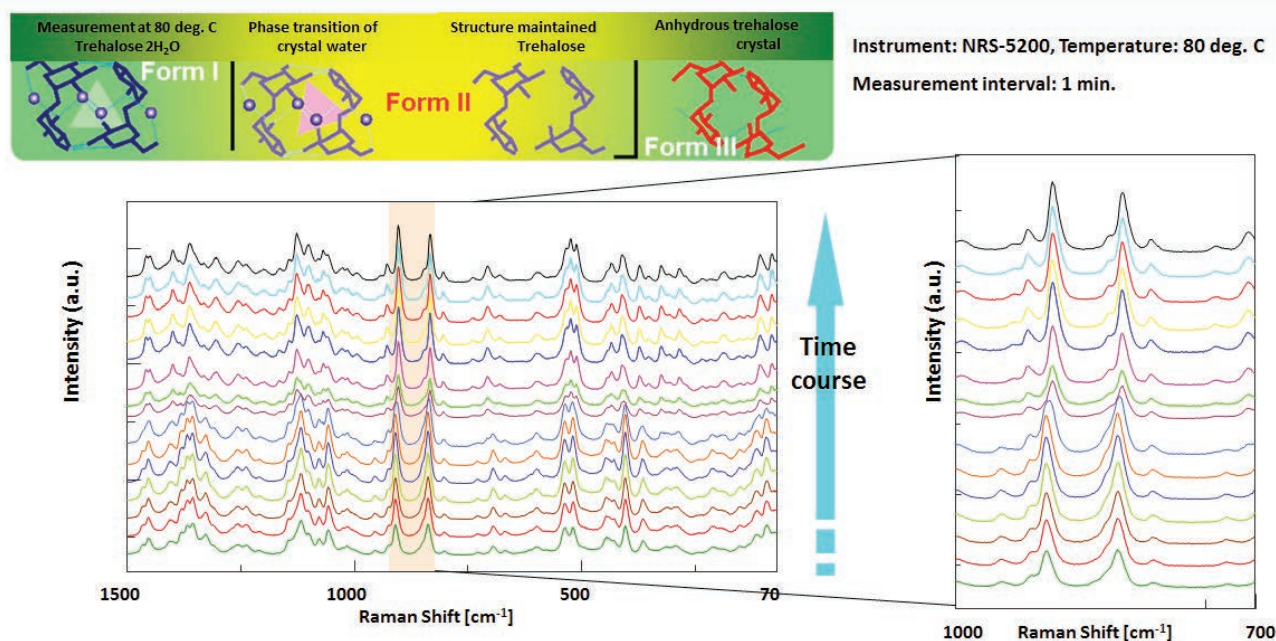
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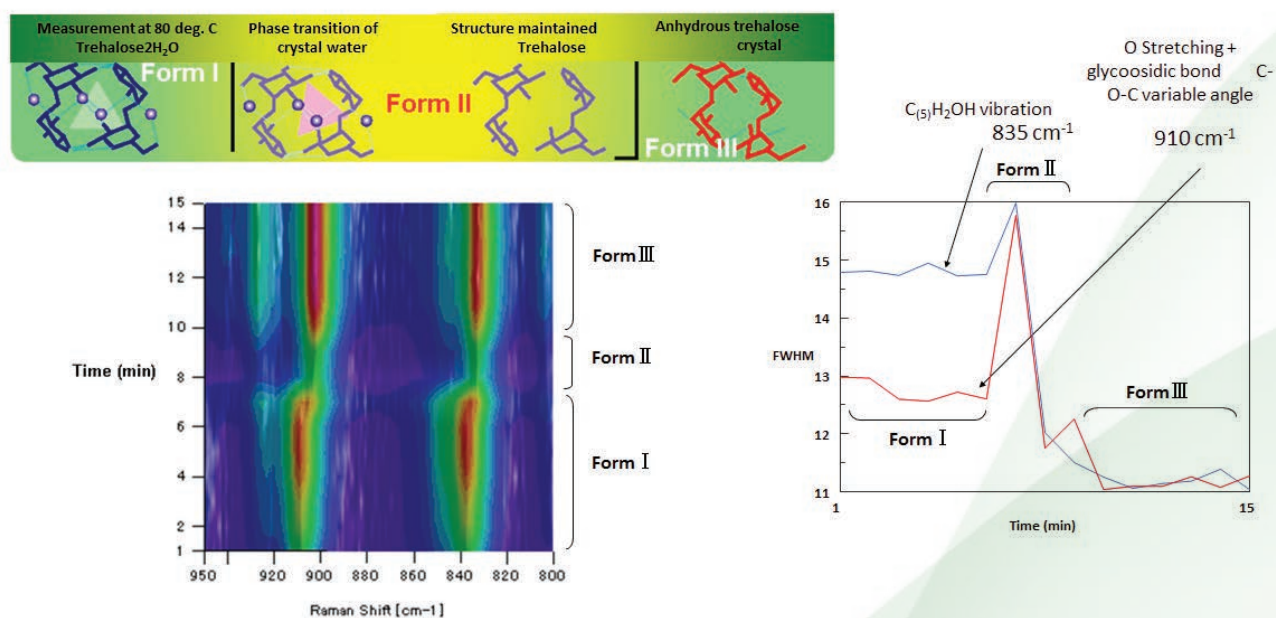
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## Real-time Monitoring of Trehalose using a Heating Stage (1)



In-situ monitoring of Trehalose 2H<sub>2</sub>O (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.

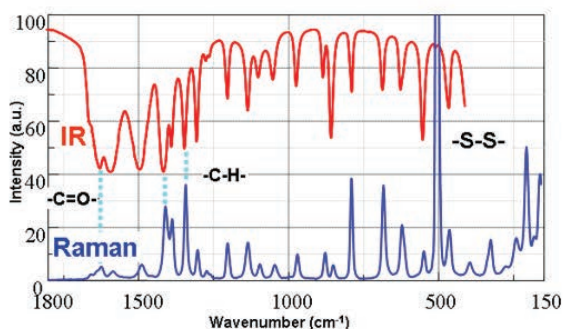
## Real-time Monitoring of Trehalose using a Heating Stage (2)



The half bandwidth and the peak shift of two peaks which demonstrate the hydration feature in the previous page were analyzed. The results indicate 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<sup>-1</sup>. 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 changes with sample melting.

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

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



IR and Raman spectra  
(L-cystine[ $-\text{SCH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$ ] $_2$ )



Micro-Raman spectrometer (NRS-5000)

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

## Low Wavenumber Measurements using a Notch Filter

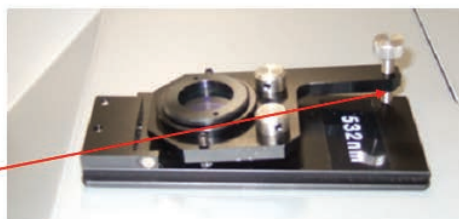
## What is a notch filter...?

A notch filter is an optical element used to remove the very strong Rayleigh scattering light from the sample measurement. 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.



- Easy replacement design

Notch filter angle can be adjusted by the user!



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  $5\text{ cm}^{-1}$  using the low wavenumber measurement unit.

NRS-5100/7100	: down to $50\text{ cm}^{-1}$
NRS-5200 with low wavenumber measurement unit	: down to $10\text{ cm}^{-1}$
NRS-7200 with low wavenumber measurement unit	: down to $5\text{ cm}^{-1}$

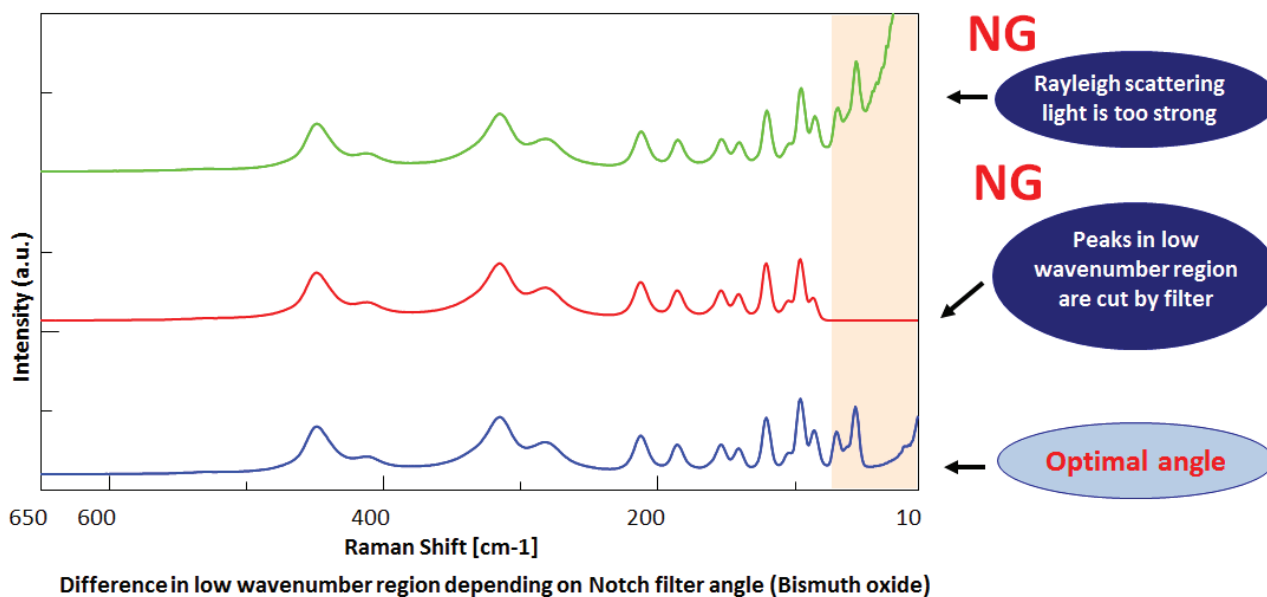
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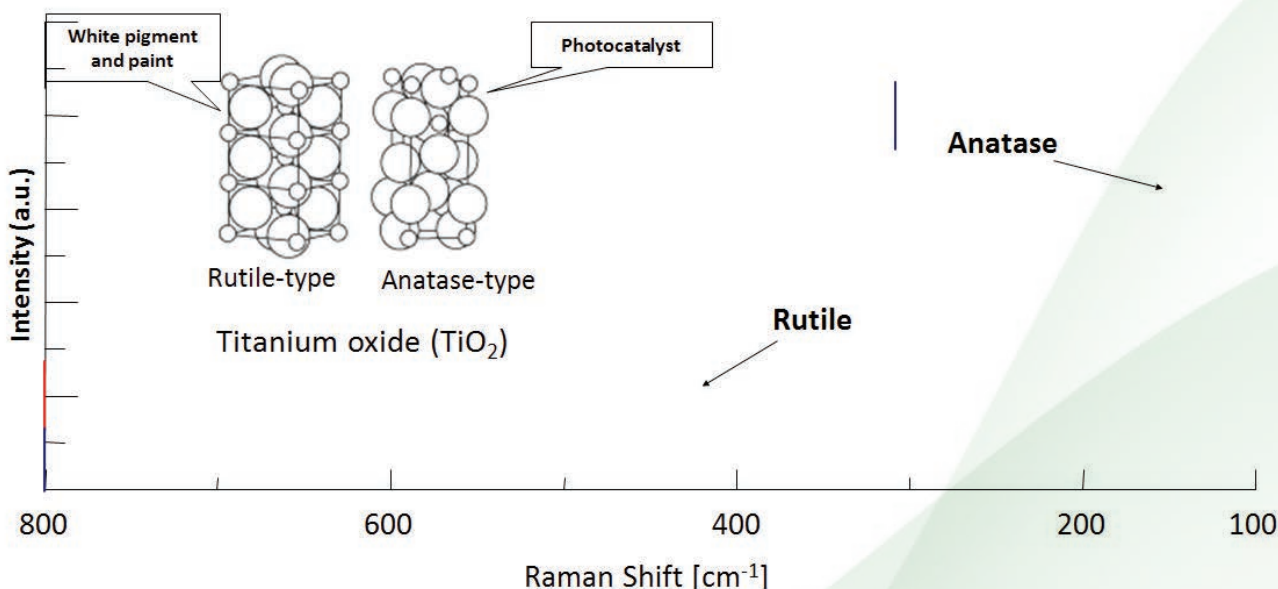
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## Low Wavenumber Measurement of Bismuth Oxide by Adjusting the Notch Filter Angle



Since peaks of minerals or lattice vibration derived from crystals are detected in the 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 the low wavenumber region, so careful optimization is necessary to adjust the angle of the notch filter depending on the sample requirements.

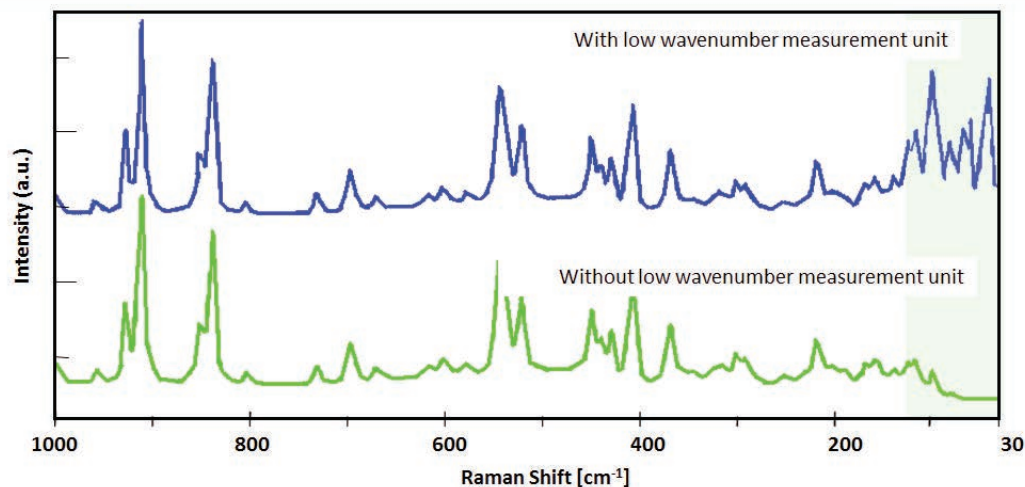
## Low Wavenumber Measurement using a Notch Filter: Titanium Oxide with Crystal Polymorphs



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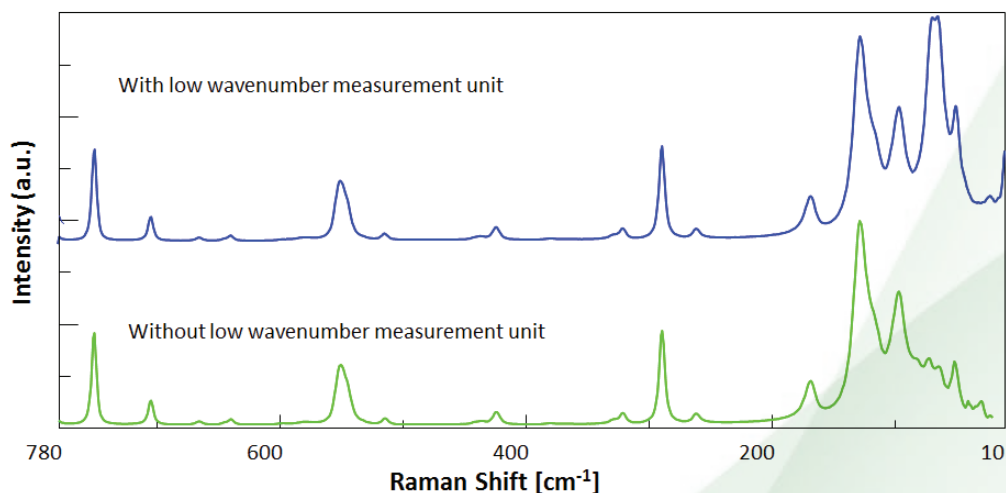
## Measurement of Trehalose using the Low Wavenumber Measurement option on the NRS-7200



NRS-7200 with low wavenumber measurement unit

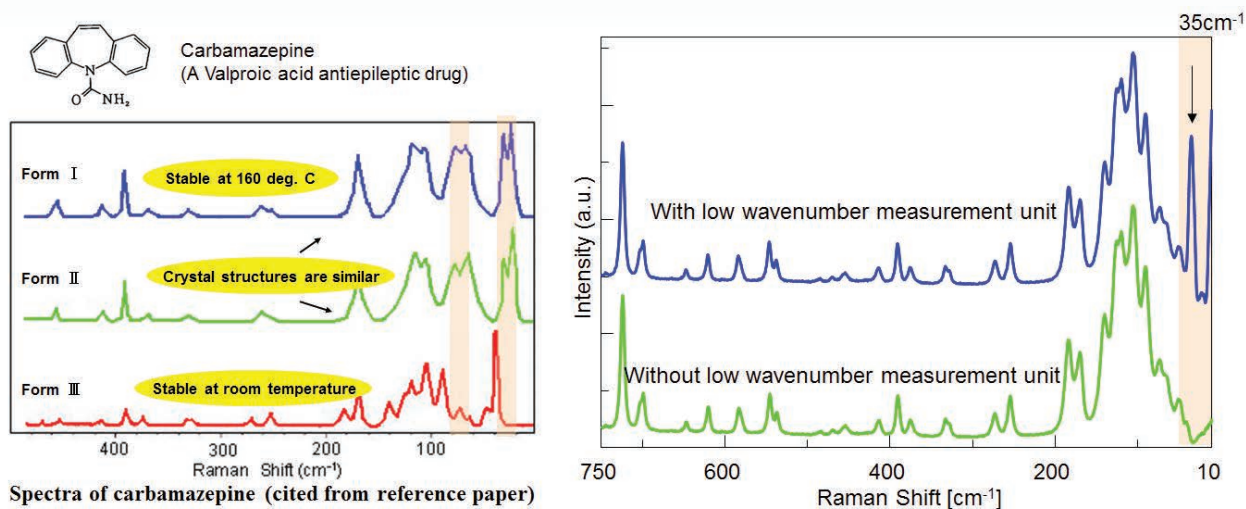
Sometimes important peaks are obscured by the notch filter when used with a single monochromator, however, the low wavenumber measurement unit fitted to the NRS-7200 enables clear measurements in the low wavenumber region.

## Measurement of Acetylsalicylic acid using the NRS-7200 Low Wavenumber Measurement Unit



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 using the option. Using the low wavenumber measurement unit, peaks for the lattice vibrations in the analgesic compound can be clearly observed and important information for crystal polymorph identification can be obtained.

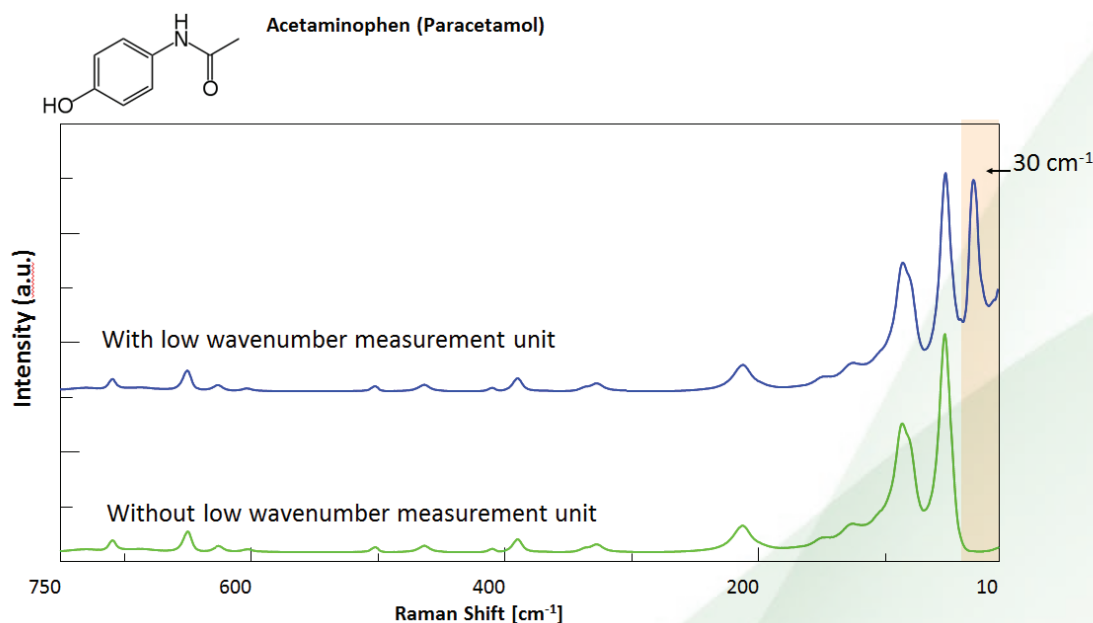
## Measurement of Carbamazepine Polymorphs using the NRS-7200 Low Wavenumber Measurement Unit



Patrick A. Anquetil et al., *J. Pharm. Sci.*, **92**, 149-160 (2003).  
A.L. Grzesiak, M. Lang, K. Kim, A.J. Matzger, *J. Pharm. Sci.*, **92**, 2260-2271 (2004).  
C.J. Strachen, S.L. Howell, T. Rades, K.C. Gordon, *J. Raman Spectrosc.*, **35**, 401-408 (2004).

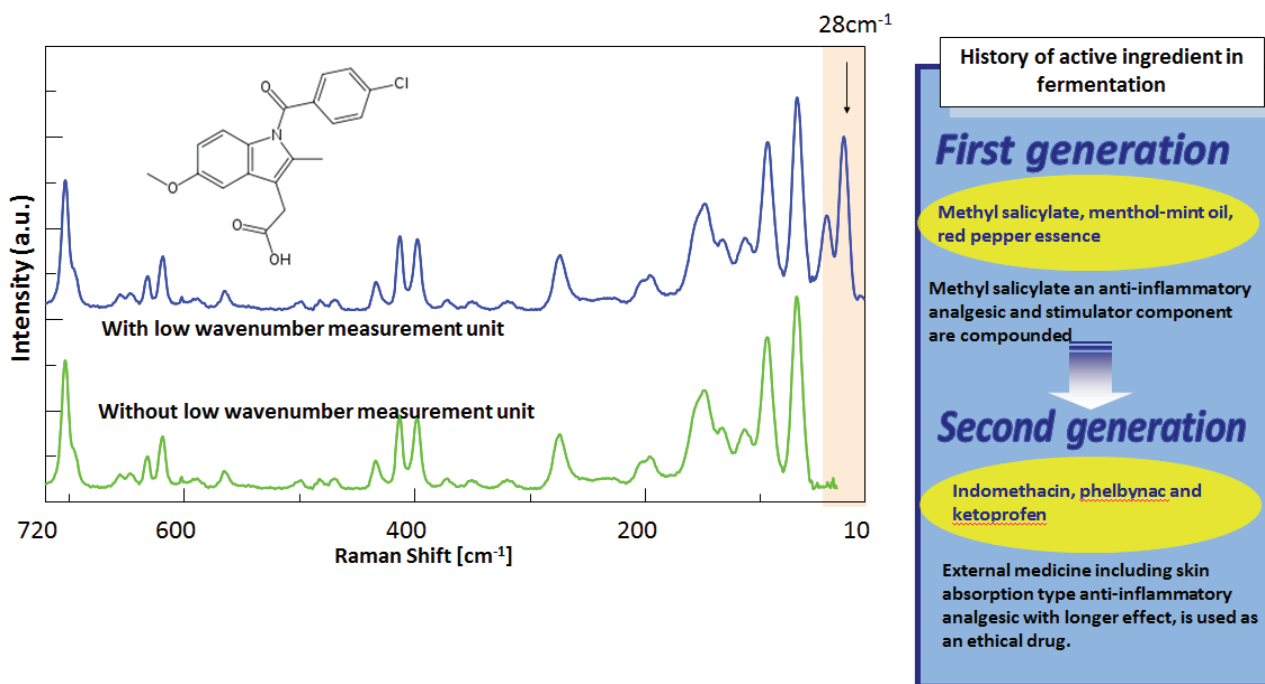
Since Raman spectroscopy can easily detect peaks in the wavenumber region below 100 cm<sup>-1</sup>, it is possible to identify the differences between Forms I and II of carbamazepine whose crystal structures are quite similar. However, the low wavenumber measurement unit is required to characterize the Form III polymorph.

## Measurement of Acetaminophen (Paracetamol) using the NRS-7200 Low Wavenumber Measurement Unit



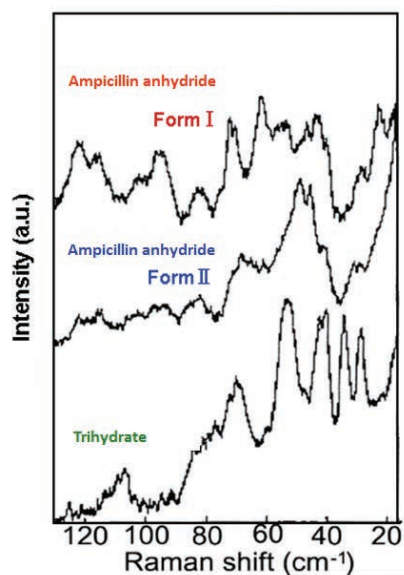
Commercially available Acetaminophen was measured using the NRS-7200. It is known that there are three crystal polymorphs of Paracetamol - Form I, II and III. The above spectra shows the Form I polymorph.

## Measurement Indomethacin Polymorphs using the NRS-7200 Low Wavenumber Measurement Unit: Patent of Crystal Polymorphs



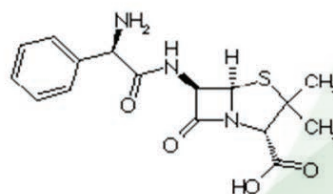
The above spectra were obtained by measuring commercially available Indomethacin using the NRS-7200 with and without the low wavenumber measurement unit. It is known that Indomethacin exists as three different crystal polymorphs: alpha (acicular crystal), beta and gamma (plate crystal). Gamma is the most stable structure followed by the alpha structure. 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.

## 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 vibrations 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 low wavenumber Raman spectra.

## Measurement Indomethacin Polymorphs using the NRS-7200 Low Wavenumber Measurement Unit: Patent of Crystal Polymorphs

### 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 directly measure commercially available medicine, suggesting use for the evaluation of final products

### Low wavenumber measurement is essential for evaluation of crystal polymorphs!

In comparison with other Raman instruments, the NRS-7200 allows ...

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

