WHITEPAPER

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Application of Low-Frequency (LF) Raman Spectroscopy to an Isoenergetic Polymorph Study

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Introduction

The importance of polymorphism in the pharmaceutical industry is well established.[i] Much literature exists describing the effect of polymorphs on efficacy,[ii] the phenomenon of polymorphism,[iii] and the analytical tools available to study polymorphism in active pharmaceutical ingredients and drug products.[iv] Standard analytical techniques in the study of polymorphism include x-ray diffraction, differential scanning calorimetry, thermogravimetry, vapor sorption analysis, vibrational spectroscopy, and solid-state nuclear magnetic resonance spectroscopy.

Typically x-ray powder diffraction (XRPD) is the most significant and first-choice method utilized to differentiate polymorphic forms of a substance, due to the fact that XRPD probes molecular packing directly, an XRPD pattern being a fingerprint of a specific three-dimensional arrangement of molecules. Other techniques probe crystalline structure indirectly, and therefore are occasionally unable to clearly differentiate polymorphs. For example, differential scanning calorimetry (DSC) discriminates by detection of different melting points, which are a consequence of different crystalline structures. Vibrational spectroscopic techniques, such as infrared (IR) and Raman spectroscopy, can sometimes be used to differentiate polymorphs based on the effects of molecular packing on molecular bond vibrational energies.

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Low-Frequency (LF) Raman Spectroscopy

Standard Raman spectroscopy probes vibrational energies in the range of about 200 to 4,000 cm^{-1}. Many vibrations of atoms about bonds occur the range from about 500 to 1700 cm^{-1}, which is often referred to as the molecular "fingerprint" region, where most C–C and C–X (X = H, N, O, S, etc.) vibrations occur. Information in a standard Raman spectrum reveals the natures of the bonds holding a molecule together and, in some cases, interactions between neighboring molecules. However, as an indirect probe, standard Raman spectroscopy frequently is insufficient for the differentiation of polymorphs.

In addition to molecular bond vibrations that occur in the fingerprint region, there exist phonon vibrations, which occur in the range of about 5 to 200 cm^{-1}. That range is known as the low-frequency (LF) or terahertz (THz) region. Phonon vibrations arise from cooperative oscillation of an ordered array of atoms at a single frequency (Figure 1). Therefore, LF Raman spectroscopy is ideally suited to the study of crystalline materials. Different polymorphic forms of the same substance would be expected to produce different LF Raman spectra.

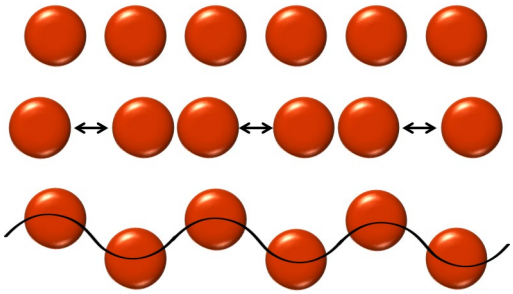


Figure 1. Depiction of phonon vibrations.

The advent of highly-efficient, narrow-band blocking filters allow construction of Raman systems that can capture signals from the LF region. Such filters, like the SureBlock™ series offered by ONDAX, remove up to 99.9999% of the Rayleigh line to within 5 cm^{-1} of the laser line. Triclinic acquired an ONDAX THz-Raman® module equipped with a SureBlock™ notch filter and an 853-nm CleanLine™ laser, which was interfaced to our Renishaw inVia Raman spectrometer. That system allows spectra to be obtained by several sample presentations, including powder samples in an NMR tube or min any container using contact, non-contact, or immersion probes. The immersion probes can be used to follow crystallizations, slurry interconversions, and two-phase, solid-liquid reactions in real time. A whole-tablet holder allows us to obtain transmission spectra of intact tablets. Additionally, a transmission Raman accessory allows collection of spectra from whole intact tablets or through the walls of vials containing powders. A further

advantage of the system is that signals from both the fingerprint and LF regions can be obtained in a single experiment.

Isoenergetic Polymorphs

Isoenergetic polymorphs have been defined as polymorphs that are "virtually indistinguishable except by means of X-ray diffraction".[v] That is a quite specific definition. In practice, polymorphs are encountered that do not precisely meet that definition but are difficult to study because of their structural similarities and consequent shortage of usable analytical techniques.

As an example, phenobarbital exists in two anhydrous polymorphic forms that are structurally similar.[vi] Polymorphs I and II of that compound were described as "energetically almost indistinguishable" because of the similarity of their solubilities, heats of solution, melting temperatures, heats of fusion, infrared spectra, Raman spectra, and even XRPD patterns. Ultimately, single-crystal x-ray diffraction studies were required to understand the structural difference of those polymorphs.[vi]

A somewhat similar situation was found for tolazamide. Only one crystalline form of tolazamide had been recognized prior to a 2015 publication.[vii] Knowing that sulfonylureas, of which tolazamide is one, are often polymorphic, the authors carried out an in-depth study leading to the identification of three polymorphs. Those were described as being "practically isoenergetic", which apparently inhibited their earlier discovery.

*Developmental Compound **A***

A study was undertaken at Triclinic Labs to understand the polymorphic nature of a compound under development as an API, referred to herein as compound **A**. Compound A is known to exist in two anhydrous polymorphic forms (**1** and **2**) and a hydrated form (**3**). The x-ray powder diffraction (XRPD) patterns of **1** and **2** are very similar (Figure 2). The pattern of polymorph **2** exhibits stand-alone, unique peaks not seen in the pattern of **1** at about 18.1, 22.0, 26.3, 29.1, and 34.1 º2θ. On the other hand, there appear to be no peaks in the pattern of **1** that are not overlapped by peaks in the pattern of **2**. The similarity of the XRPD patterns of the anhydrous polymorphs suggests that they are structurally very alike, i.e. isoenergetic. The XRPD pattern of **3** is unique.

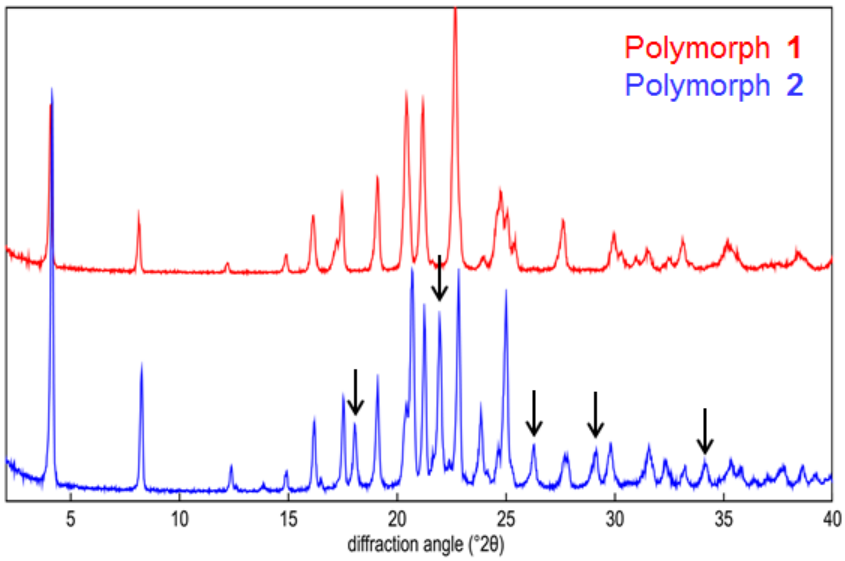


Figure 2. Overlay plot of XRPD patterns of polymorphs **1** and **2**. Arrows indicate unique, free-standing peaks in the pattern of **2** not seen in the pattern of **1**.

Polymorphs **1** and **2** are indistinguishable by differential scanning calorimetry, thermogravimetry, infrared spectroscopy, and standard Raman spectroscopy. For example, Raman spectra collected on a Nicolet model 6700 FT Raman spectrometer are shown in Figure 3. The spectra are essentially identical.

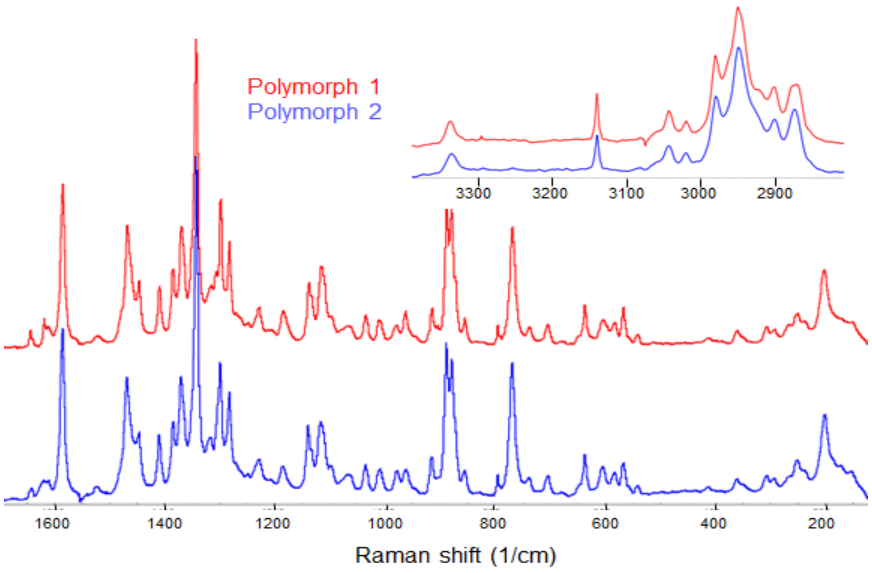


Figure 3. Overlay plot of the Raman spectra of polymorphs **1** and **2**.

The drug product that contained compound **A** consisted of undissolved **A** suspended in an aqueous solution of excipients. It was noticed that crystal shapes and particle sizes were changing during storage of drug product. It was thus important to understand the polymorphic form(s) of **A** present in the drug product and their relative thermodynamic stabilities. XRPD data of bulk starting material and bulk material from old drug products showed that initially-utilized **1** converted to **2** over time. However, the extent of conversion could not be easily determined because the only discriminating analytical technique, XRPD, cannot be used to visually discriminate pure **2** from mixtures of **1** and **2**.

Polymorphs **1** and **2** of compound **A** are easily differentiated using LF Raman spectroscopy (Figure 4). As expected, Stokes transitions are more intense than anti-Stokes transitions. Expanded views of the Stokes spectra of polymorph **1**, polymorph **2**, and the hydrate are shown in Figure 5. As shown in that figure, each crystalline form exhibits unique peaks. Even more important to the study of compound **A**, LF Raman spectroscopy can qualitatively differentiate mixtures of polymorphs **1** and **2** from samples of pure polymorph **2** (Figure 6).

It is interesting to note that the lowest phonon bands for polymorphs **1** and **2** occur at 23.0 and 14.5 cm^{-1}, respectively. Since the crystal lattices of **1** and **2** are quite similar, it is expected that those bands represent the same phonon mode in each structure. It is important to note that crystal lattice vibrational frequencies can be no higher than the size of the smallest unit of the lattice, which is the unit cell.[viii] Given that the unit cells are the same except for doubling of the length of the *c* axis in **1** compared to **2**,[ix] it is reasonable that the observed frequency of that phonon mode is approximately doubled in **1** compared to **2**.

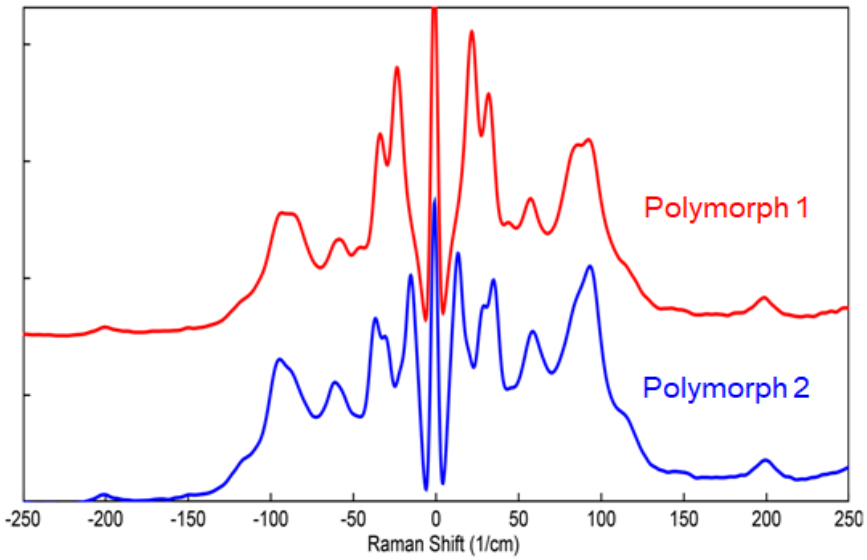


Figure 4. Overlay plot of the Stokes and anti-Stokes regions of the LF Raman spectra of **1** and **2**.

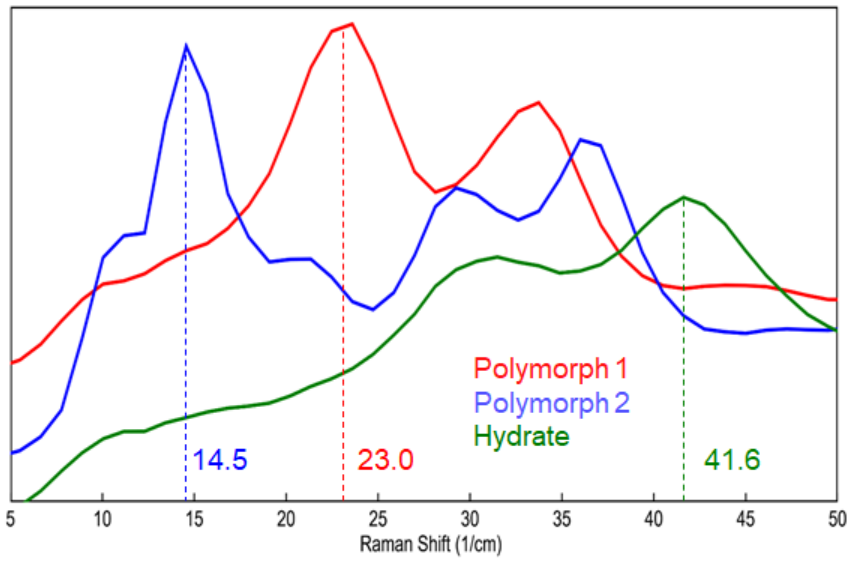


Figure 5. Overlay plot of expanded Stokes regions of the LF Raman spectra of **1, 2** and **3**. Unique phonon peaks for each structure are indicated by the dashed lines.

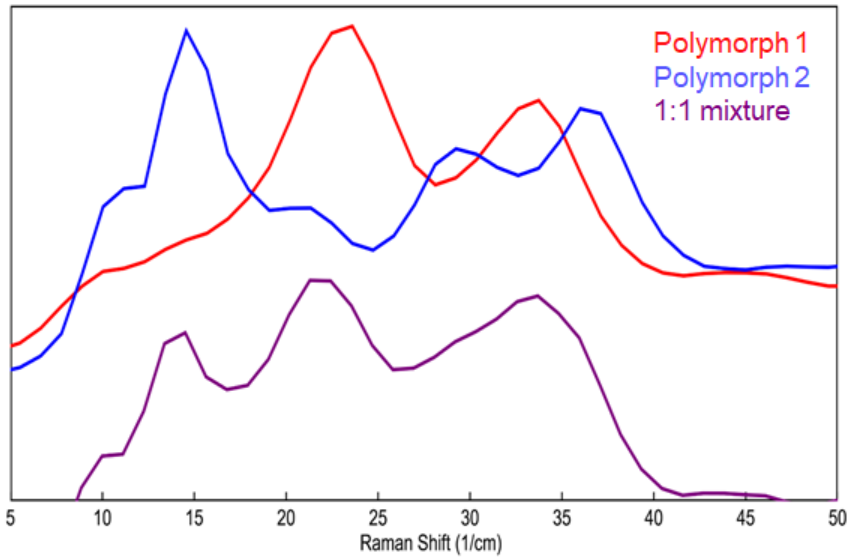


Figure 6. Overlay plot of the LF Raman spectra of **1**, **2**, and a mixture having an approximate composition of 50% **1** and 50% **2**.

The fact that polymorph **2** was more thermodynamically stable than polymorph **1** between −15 and 40 ° was determined using simple competitive slurry experiments and LF Raman spectroscopy. Saturated solutions of compound **A** in three solvent systems were treated with approximately equal portions of polymorphs **1** and **2**. The resulting

slurries were kept at three temperatures and agitated for seven days. LF Raman spectroscopy clearly showed the presence of polymorph **2** in the solid portions of the mixtures.

Since the drug product contains **A** in suspension, it was of interest to determine if polymorphic interconversion would occur in the solid state. A solid mixture containing both polymorphs **1** and **2** was kept at 40 ºC and 75% relative humidity (RH) for 14 weeks. Although slow, conversion of **1** to **2** does occur under those conditions (Figure 7).

The findings described above were both consistent with the changes in drug product during storage resulting in slow conversion of polymorph **1** to polymorph **2**. Consequently, conversion could be studied in real time using the LF Raman system equipped with an immersion probe.

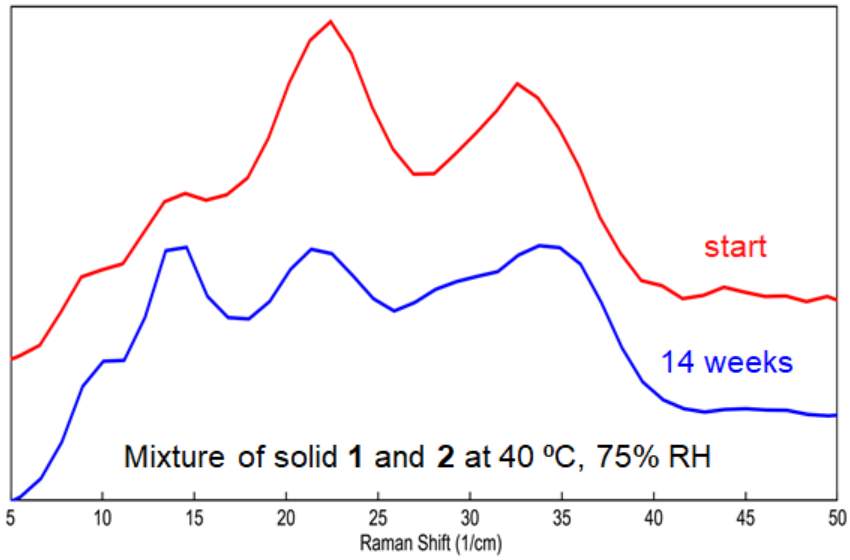


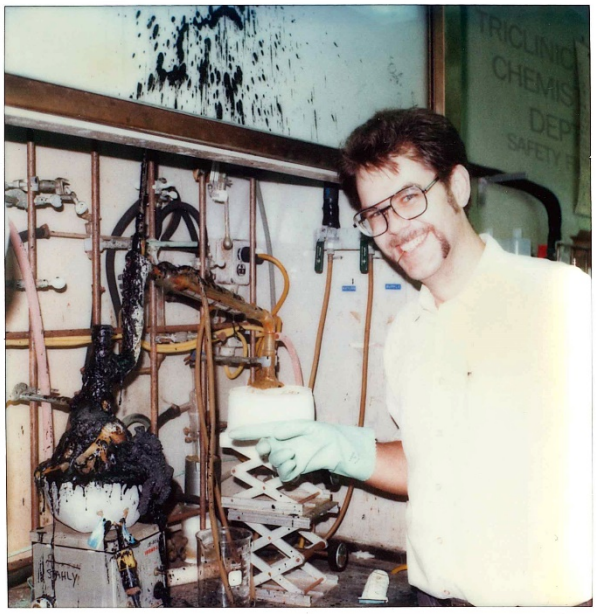
Figure 7. Overlay plot of the LF Raman spectra of a mixture of solid polymorphs **1** and **2** kept at 40 ºC and 75% relative humidity for 14 weeks.

Conclusion

LF Raman spectroscopy allowed rapid, visual, qualitative differentiation of isoenergetic polymorphs **1** and **2**, and mixtures thereof. Spectra were easily acquired and data interpretation was quite simple. Knowing the nature of the conversion, the drug developers designed a crystallization process affording polymorph **2**. Use of that form provided a stable drug product.

About the Author

Dr. Stahly is Chief Operating Officer of Triclinic Labs. Dr. Stahly has over 30 years of experience in the specialty and pharmaceutical chemical industries. Since obtaining a Ph.D. in Organic Chemistry from the University of Maryland, he held positions of increasing responsibility at the Ethyl (now Albemarle) Corporation, was Chief Operating Officer and Chief Scientific Officer of SSCI, Inc., and was Vice President of Scientific Operations of Aptuit. His expertise includes process organic chemistry, crystallization, solids analysis, X-ray diffraction, pharmaceutical preformulation, and chiral chemistry.
He is an inventor of 44 US patents and author of 33 publications, including peer-reviewed papers and book chapters. In addition, Dr. Stahly has lectured extensively throughout the world and has taught numerous courses on solid-state chemistry.



Dr. Stahly completes his first successful tert-Butyl Ester decomposition reaction.

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